Transfusion Medicine, 2006, **16**, 79–83 doi: 10.1111/j.1365-3148.2006.00652.x

ORIGINAL ARTICLE

Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country

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Received 17 October 2005; accepted for publication 4 January 2006

SUMMARY. Indigenous hepatitis E is increasingly recognized in developed countries, where it may be a zoonosis. We describe the first case of transfusion-transmitted hepatitis E in the UK from a blood donor who had no history of recent travel abroad.

Follow-up of the donor and recipients of the blood products was carried out using serological and molecular techniques.

Acute hepatitis E was transmitted to one of two recipients. The infected patient would have received a larger volume of the donor's plasma. HEV subgenomic sequences carried by the donor and recipient were identical.

This is the first case of post-transfusion hepatitis E in the UK. Secondary transmission of hepatitis E indigenous to a nonhyperendemic country may occur by blood transfusion. It is important that blood donors inform the transfusion service of all post-donation illnesses so that appropriate interventions can take place.

Key words: hepatitis E, 'post-donation illness', 'transfusion-transmitted infection'.

Hepatitis E has been characterized by outbreaks of acute hepatitis in developing countries associated with contaminated drinking water (Skidmore *et al*., 1992; Harrison, 1999; Krawczynski *et al*., 2001). Sporadic cases in developed countries have been associated with travel abroad to countries in which hepatitis E is hyperendemic (Skidmore & Sherratt, 1996; Schlauder *et al*., 1999; Schwartz *et al*., 1999).

However, evidence is emerging of zoonotic spread of hepatitis E from pigs to humans, which is supported by molecular characterization of pig and human virus (Zaaijer *et al*., 1993; Meng *et al*., 1997; Halbur *et al*., 2001; Banks *et al*., 2004). The suggested routes of transmission involve consumption of pig liver or close environmental contact with farm animals or meat products (Hsieh *et al*., 1999; Yazaki *et al*., 2003).

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Molecular characterization shows that strains from hyperendemic countries tend to belong to genotypes 1 and 2, while those from sporadic cases in developed countries belong to genotypes 3 and 4 (Takahashi *et al*., 2003; Ijaz *et al*., 2005). There is probably an underestimate of the numbers of cases of hepatitis E in the UK, as travel abroad has been used as a selection criterion for testing. However, recent studies have shown that there is significant transmission of indigenous HEV (Ijaz *et al*., 2005).

Transfusion transmission of a variety of viruses can occur if a donor presents for donation in the incubation phase of the illness, and for this reason, blood donors are encouraged to inform the Blood Service of any infection they acquire shortly after they have donated blood. Transfusion-transmitted Hepatitis E may be expected in endemic areas, and retrospective and prospective studies have confirmed this (Khuroo *et al*., 2004). Two studies from Japan reported two independent case reports of transfusion-transmitted hepatitis E, one in four dialysis patients discovered through retrospective testing (Mitsui

et al., 2004) and the other in a patient who had undergone open heart surgery and later presented with an acute hepatitis (Matsubayashi *et al*., 2004).

We describe here a case of transfusion-transmitted hepatitis E from an UK blood donor who had no history of recent travel abroad.

PATIENTS AND METHODS

Patients

The index donor is a healthy male in his 40s who had given blood for many years. He provided no history of travel abroad or of contact with anyone either with a history of travel or with recent acute hepatitis. He had never worked in the meat industry and had not recently visited any farms or pig-rearing establishments. He gave a donation on 'day 1', which was processed into platelets and optimal-additive red cells. The plasma was discarded. A platelet preparation is a pool produced from four donors and the pooled platelets are suspended in the plasma from one of the donors. These platelets were not suspended in the index donor's plasma. The donor became ill with a nonspecific 'influenza-like' illness 14 days later, and 10 days later, he became jaundiced. He contacted the Blood Service about his illness, but the components from his donation had already been used. The platelets had been given to one patient (patient 1) and the red cells to another (patient 2). Testing of blood from the donor showed that the infection was neither acute hepatitis A nor B, thereby eliminating the possibility of offering the patients immediate specific immune prophylaxis. The clinicians managing the patients were notified of the nature of the donor's illness and were advised that a better assessment of the risk to their patients would follow further investigation of the donor's illness including nucleic acid testing of serum archived from the donation.

Patient 1. Patient 1 was a 55-year-old female with primary biliary cirrhosis and choledocholithiasis who received a unit of platelets (on day 5) prior to undergoing endoscopic retrograde cholangiography.

Patient 2. Patient 2 was a 65-year-old male with a history of a successfully treated B-cell lymphoma of the testicle. He presented with a 6-week history of cerebellar symptoms. MRI brain scan showed a lobulated solitary enhancing right cerebellar mass. The cerebellar mass was assessed as unsafe to biopsy so the patient was treated empirically with the IDRAM regime (involving orally administered idarubicin, cytarabine and methotrexate, intravenous dexamethasone, and intrathecal methotrexate and cytarabine). Repeat MRI scanning after two courses of treatment showed resolution of the mass. A total of four courses were given. During the course of this treatment, the patient became anaemic requiring transfusions of concentrated red cells. The implicated donation was given on day 18 after the donation, 5 days before the donor became jaundiced and after the recipient had had three courses of chemotherapy.

Methods

Diagnostic tests on the donor and patients were applied using standard serological techniques. Hepatitis E serology was carried out using IgG and IgM enzyme-linked immunosorbent assays (ELISAs) (Genelabs, Genelab Technologies Inc., Redwood City, CA, USA). Reactive samples were tested for HEV RNA using a previously described method using nested PCR of reverse transcribed cDNA derived from open reading frame 2 of the HEV genome (Meng *et al*., 1997). A 280-bp region of ORF2 was analysed. Through phylogenetic analysis, sequences generated from this study were compared with other UK indigenous HEV strains which had been previously identified (Ijaz *et al*., 2005), as well as other genotype 1, 2, 3 and 4 sequences retrieved from GenBank.

RESULTS

The donor was found seronegative for markers of acute hepatitis A and B and infection by hepatitis C virus, Epstein–Barr virus and cytomegalovirus but strongly seropositive for anti-HEV IgM and IgG antibodies. At the time of the onset of jaundice, the alanine amino transferase (ALT) level was 2050 IU L^{-1}, alkaline phosphatase 501 IU L^{-1} and bilirubin 101 IU L^{-1}. The donor's acute-phase serum sample and the donation archive carried HEV RNA whose nucleotide sequences showed that they belonged to genotype 3 HEV. The finding of the presence of HEV RNA in the archive sample suggested that components of the donation presented a risk of HEV infection to both patients.

Serum samples obtained from the recipients showed that both were negative for antibody to hepatitis E and both were therefore considered susceptible to infection.

Patient 1 had received platelets that were not suspended in the implicated donor's plasma, and the platelet preparation was therefore considered to carry a low risk of infection. The other three platelet

donors were subsequently tested and found to be negative for HEV IgG. Follow-up testing at 84 days after administration of the blood product showed no appearance of anti-HEV antibodies.

Test results for patient 2 are shown in Fig. 1. Patient 2 was followed up more closely, as he was under continuing care of the haematologists and considered to be at a greater risk of infection. HEV RNA was detected at day 34 (5 weeks – no earlier archived samples were available for RNA testing) and became undetectable by day 89 (12 weeks) post-transfusion. Sequence analysis showed the strain carried by him to be identical to that from the implicated donor. The sequences generated clustered closely with genotype 3 strains isolated from the UK. Recently isolated UK genotype 3 strains have an 80–100% sequence homology at the nucleotide level. Moreover, 100% homology is unusual and has been observed in these transfusion-related isolates and in a small cluster of five cases; however, the transfusion-related strain clusters away from this group. The serum taken on day 67 (9 weeks) post-transfusion was equivocally reactive for anti-HEV IgM (test/cut off optical density ratio: 1·32). This reactivity became stronger in samples taken on day 75 (ratio: 2·82) and day 89 (ratio: 5·06), confirming an active infection and ruling out passive transfusion of HEV RNA. Liver biochemistry results showed a peak of abnormality (maximum AST 780 IU L^{-1}) at day 67 (9 weeks) returning to normal by day 89 (13 weeks). The patient remained asymptomatic apart from a mild jaundice. Samples taken up to 132 days (19 weeks) post-transfusion showed a falling anti-HEV IgM reactivity but without detectable anti-HEV IgG.

DISCUSSION

This is the first case of transfusion-transmitted hepatitis E occurring in a nonhyperendemic country. Transfusion-transmitted hepatitis E has been reported from Saudi Arabia (Khuroo *et al.*, 2004) and Japan (Matsubayashi *et al.*, 2004; Mitsui *et al.*, 2004). Hepatitis E has been thought to be a rare infection in the UK being usually associated with recent travel abroad (Health Protection Agency archive). Accordingly, the risk of a UK donor carrying circulating HEV at the time of donation is estimated to be very low.

In this case of post-transfusion hepatitis E, blood had been donated 3 weeks before the onset of jaundice. It has been estimated that patients are viraemic from about 2–3 weeks before the peak ALT level, with peak ALT levels occurring at about 5 weeks post-infection (Krawczynski *et al.*, 2001). The donation proved to carry HEV RNA, confirming the presence of viraemia before the onset of jaundice and therefore presenting a risk of transmission.

Both recipients were susceptible to hepatitis E, but only one became infected. Although the majority of plasma from the index donation was discarded, the infected recipient had received optimal-additive red cells, which would have included up to 30 mL of plasma. The patient who received the platelets would have received a maximum of 3–4 mL of the index donor plasma, giving an exposure 10 times lower. The possibility that the suspending plasma may have 'neutralized' the virus was unlikely as the other donors lacked antibody to hepatitis E. Patient 2 was observed to be viraemic 34 days (5 weeks) after

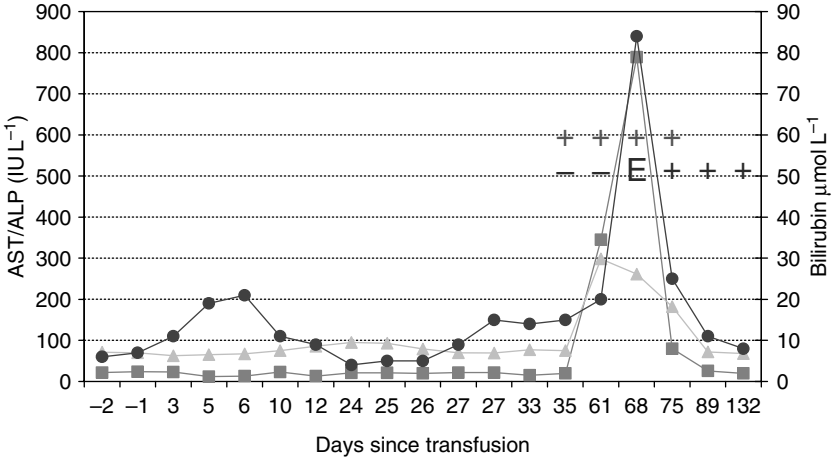

Fig. 1. Patient 2. ■, aspartate amino transferase level (IU L^{-1}); ▲, alkaline phosphatase level (IU L^{-1}); ●, bilirubin (IU L^{-1}); +, HEV RNA detected; +, HEV IgM detected; –, HEV IgM not detected; E, equivocal for HEV IgM.

transfusion and remained viraemic for 7 weeks (till day 89) with the AST peaking at about the 9th week and anti-HEV IgM appearing from the 10th week. As the patient remained well during this episode, it is not possible to give an exact date of onset of illness. Although ALT values are more commonly used in monitoring viral hepatitis, we were fortunate that patient 2 was being monitored as a consequence of his chemotherapy, which gave us a unique series of AST values both prior to and following his transfusion.

The onset of illness in the case described by Matsubayashi *et al.* (2004) was about 6 weeks post-transfusion, with the serum ALT level of 1595 IU L^{-1} (AST 1727 IU L^{-1}). In this case, both anti-HEV IgM and IgG antibody were reactive by 5 weeks, indicating an 'incubation period' which was almost half that for patient 2. It is likely that this difference is related to the infectivity of the products transfused. The Japanese patient who became infected was transfused with fresh-frozen plasma.

Patient 2 even on follow-up to 19 weeks did not produce any anti-HEV IgG. It is possible that his underlying lymphoma or his cytotoxic treatment resulted in delayed immunoglobulin isotype switching. Delay in the reconstitution of the immune system in children after chemotherapy for acute lymphocytic leukaemia has been described, and it is recommended that routine childhood immunizations be delayed until 6 months post-therapy (Ek *et al.*, 2005).

Further studies of Japanese blood donors have revealed an anti-HEV seroprevalence of 3·7%. Of 343 donors tested, three could be identified to be anti-HEV IgM and HEV RNA seropositive (Fukuda *et al.*, 2004). High levels of HEV IgG were found to be associated with raised ALT and AST levels. One of the donors showed a serum ALT level of 966 IU L^{-1} (AST 815) and the other two showed levels of 61 (AST 22) and 62 IU L^{-1} (AST 37). They showed no signs or symptoms of hepatitis. All were infected by HEV belonging to genotype 3, which were closely related to each other and to recently isolated strains in Japan.

HEV of genotype 3 is increasingly being described in patients with no history of travel to hyperendemic areas. In a study of 'undiagnosed' sporadic acute hepatitis in southwestern France, 16 of 46 patients with anti-HEV IgG proved to be viraemic for hepatitis E RNA whose sequences clustered with those of genotype 3. Patients diagnosed with hepatitis E showed a male preponderance and were generally in older age groups; the only risk factor for this group of patients was their residence in a rural location (Mansuy *et al.*, 2004). Recent UK studies have also shown HEV genotype 3 to be associated with sporadic cases of hepatitis and preponderance of infections in older men has been described. (Ijaz *et al.*, 2005).

Post-transfusion hepatitis in the UK is now relatively rare even with enhanced surveillance through the Serious Hazards of Transfusion (SHOT) reporting system (SHOT 2003). An incubating hepatitis E infection was not suspected in our donor at the time of his donation and would not have been expected in the recipients of his donation had the donor not become jaundiced. Therefore, we cannot be sure how often this complication might occur in the UK. A 'near miss' possible transfusion transmission of hepatitis E was described recently from Hong Kong. The donor presented 1 week after donation with an illness which included vomiting, diarrhoea and jaundice with an ALT level of 8000 IU mL^{-1} (AST level not reported). The donor was able to inform the transfusion service before the products (red cells and fresh-frozen plasma only) were used (Lee *et al.*, 2005). That case endorsed the usefulness of giving donors clear instructions to inform the transfusion service of any post-donation illness. However, platelet transfusions will always present a risk, as they must be used within a few days of collection.

Our case reveals another facet of the consequence of sporadic hepatitis E indigenous to the UK. Hepatitis E should now be considered when investigating post-transfusion hepatitis and post-donation illnesses. Donor screening protocols may not need to be changed to include hepatitis E and other transient infections. However, the results of enhanced surveillance of indigenous hepatitis E currently being undertaken in the UK might shape future policy. In the meantime, it must be stressed to donors that any and all post-donation illnesses must be reported to the transfusion services. The NBS operates a 24-h helpline for donors to report post-donation problems.

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