Absence of persistent Hepatitis E virus infection in antibody-deficient patients is associated with the transfer of antigen neutralising antibodies from immunoglobulin products

Authors

Mike Ankcorn[1,2], Fernando Moreira[3], Samreen Ijaz[1], Andrew Symes[3], Matthew S Buckland[3,4], Sarita Workman[3], Fiona Warburton[5], Richard S Tedder[1,2,6] & David M Lowe[3,4].

Affiliations

1 Blood Borne Virus Unit, Virus Reference Department, National Infection Service, Public Health England, Colindale, London, United Kingdom.

2 Transfusion Microbiology, National Health Service Blood and Transplant, London, United Kingdom.

3 Department of Clinical Immunology, Royal Free London NHS Foundation Trust, London, United Kingdom.

4 Institute of Immunity and Transplantation, University College London, Royal Free Campus, London, United Kingdom.

5 Statistics and Modelling Economics Department, Public Health England, Colindale, London, United Kingdom.

6 Division of Infection and Immunity, University College London, Gower Street, London, United Kingdom.

Brief Summary

A cohort of antibody deficient patients were tested for active HEV infection. None of 245 patients were viraemic, however antigen-neutralising anti-HEV IgG was detected in patients sera and immunoglobulin products. Immunoglobulin replacement may protect these patients from persistent HEV infection.

Footnotes

Authorship:

Ankcorn MJ – Study concept and design, Clinical data collection, data analysis, sample testing, drafting of manuscript.

Moreira F – Recruitment of patients, Clinical data collection, review of final manuscript.

Symes A – Recruitment of patients, Clinical data collection, review of final manuscript.

Workman S - Recruitment of patients, Clinical data collection, review of final manuscript.

Warburton F – Statistical guidance and interpretation, review of final manuscript.

Ijaz S - Study concept and design, data interpretation, review of final manuscript.

Tedder RS - Study concept and design, data interpretation, review of final manuscript.

Lowe DM - Study concept and design, clinical data collection, data interpretation, drafting of manuscript and review of final manuscript.

Conflicts of Interest:

The authors declare the following conflicts of interest:

DML has received grants from UCL Biomedical Research Centre, grants from Rare Diseases Foundation and has received travel and subsistence costs for consultancy work from CSL Behring. SW has received travel and subsistence costs to attend educational meetings from CSL Behring, Octapharma, Grifols, Biotest, BPL, LFB, an educational grant from CSL Behring and honorariums

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Corresponding author:

Dr Michael J Ankcorn

Clinical Research Fellow in Virology (NHS Blood and Transplant), Blood Borne Virus Unit, Virus Reference Department, National Infection Service, Public Health England, London, NW9 5EQ, United Kingdom.

0208 327 6422.

Michael.Ankcorn@phe.gov.uk

Alternate corresponding author:

Dr David Lowe

Consultant clinical immunologist, Department of Clinical Immunology, Royal Free London NHS Foundation Trust, London, NW3 2QG, United Kingdom.

020 7794 0500.

d.lowe@ucl.ac.uk

Abbreviations

ALT, alanine aminotransferase

Anti-HEV IgM, IgM antibody to hepatitis E virus

Anti-HEV IgG, IgG antibody to hepatitis E virus

CLL, chronic lymphocytic leukaemia

CVID, common variable immune deficiency

ELISA, enzyme-linked immunosorbent assay

G3, Genotype 3

HEV, Hepatitis E virus

HEV-Ag, Hepatitis E virus antigen

HSCT, haematopoietic stem cell transplant

HIV, human immunodeficiency virus

IU, international units

IQR, interquartile range

IV, intravenous

IVIG, intravenous immunoglobulin

LLN, lower limit of normal

MGUS, monoclonal gammopathy of uncertain significance

ML, millilitre

NHP, normal human plasma

NHSBT, National Health Service Blood and Transplant

NIBSC, National Institute for Biological Standards and Control

NK cells, natural killer cells

OD, optical density

ORF2, open reading frame 2

PHE, Public Health England

RNA, ribonucleic acid

RTX, rituximab

SC, subcutaneous

SCIG, subcutaneous immunoglobulin

ULN, upper limit of normal

WHO, World Health Organisation

XLA, X-linked agammaglobulinaemia

Abstract

Background

Persistent hepatitis E virus (HEV) infection is described in a number of immunosuppressive conditions. We aimed to determine the risk of persistent HEV infection in patients with primary or secondary antibody deficiency.

Methods

Two hundred and forty five antibody deficient patients on regular immunoglobulin replacement were tested for HEV RNA and anti-HEV IgG. Immunoglobulin products and plasma from nine antibody-deficient patients pre- and post-IVIG, five recently treated patients with persistent HEV infection and five healthy patients recovered from acute HEV infection were analysed for anti-HEV IgG and for antibody reacting with HEV antigen (HEV-Ag).

Results

No antibody deficient patient had detectable plasma HEV RNA. Anti-HEV IgG was detected in 38.8% of patients. All ten immunoglobulin products tested contained anti-HEV capable of neutralising HEV-Ag. Plasma samples following IVIG infusion demonstrated higher anti-HEV IgG and neutralising activity compared with pre-IVIG samples. Neutralising activity was similar to healthy patients with recent acute HEV infection.

Conclusion

The risk of persistent HEV infection in patients with antibody deficiency appears extremely low. This may be due to passive seroprotection afforded by the ubiquitous presence of anti-HEV in immunoglobulin replacement products.

Keywords: hepatitis E virus, chronic infection, antibody deficiency, immunoglobulin, IVIG.

Background

Hepatitis E virus (HEV) is a small, non-enveloped, single-stranded RNA virus comprising four major genotypes which infect humans [1]. Genotype 3 (G3) HEV infection is predominantly a foodborne zoonosis which has emerged as the leading cause of acute viral hepatitis in Western Europe [2].Transmission also occurs through substances of human origin including blood transfusion and organ transplantation [3-5]. Typically G3 HEV infections are self-limiting but can persist in the context of solid organ transplantation, HIV infection, haematological malignancy and auto-immune disease [6-11]. Persistent HEV infection is frequently undiagnosed due to being pauci-symptomatic: the elevated liver enzymes associated with infection are often attributed to other conditions [12].

Antibody deficiency is characterised by low immunoglobulins or the functional failure of immunoglobulins. This may be caused by a primary immunodeficiency syndrome or secondary causes including haematological malignancy and medications [13]. Typically this manifests as recurrent infections with encapsulated bacteria but also with a range of viral, fungal or protozoan pathogens. For example, prolonged rhinovirus infections, severe hepatitis C virus and enterovirus infections are described in patients with primary antibody deficiency, whilst norovirus can lead to severe prolonged enteropathy in common variable immune deficiency (CVID) [14-17]. Immunoglobulin replacement therapy is generally recommended for serious immune deficiency (eg CVID or X-linked agammaglobulinaemia (XLA)), significantly low IgG (<4g/L), the presence of end-organ disease (especially bronchiectasis) or if the patient is suffering recurrent bacterial infection despite antibiotic prophylaxis.

Few studies have addressed the prevalence of persistent HEV infection in these patients. A study of 73 patients with CVID in Germany in 2012 and more recently, a study of 27 patients with primary antibody deficiency and abnormal liver enzymes in the UK, found no evidence of persistent HEV infection [13, 18]. However the risk of HEV acquisition in Western Europe has risen in recent years [2].

Our aim was to determine whether there was evidence of persistent HEV infection in a cohort of patients with primary and secondary antibody deficiency on immunoglobulin replacement therapy at a large teaching hospital in London. We evaluated immunoglobulin products directly and a subset of patients pre- and post-IVIG infusion for measurable antibody to HEV including antibody reactive with HEV open reading frame 2 (ORF2) antigen. These samples were compared with plasma from patients in recovery from acute and persistent HEV infection.

Materials and Methods

Patients and products

For the cross-sectional testing, patients were eligible if they had a diagnosis of antibody deficiency requiring regular immunoglobulin replacement therapy under follow-up at the Royal Free Hospital, London, UK. Fifty eight patients had stored plasma samples from a previous study in 2015, a further 187 patients were prospectively recruited [19]. Pre- and post- immunoglobulin infusion analysis of HEV antibodies was undertaken in nine patients. Ten immunoglobulin products (and different batches for three products) were also analysed for HEV antibodies.

For investigation of HEV-Ag neutralisation activity, plasma from five recovered acute HEV cases and five persistent HEV cases following viral clearance (range 11-23 weeks after plasma clearance) on ribavirin therapy were selected. All ten samples were reactive for anti-HEV IgM and IgG and negative for HEV RNA and HEV-Ag.

Anti-HEV IgG data from blood donor samples archived prior to HEV infection from a previous study were used to define reactivity of plasma samples from persons known to be susceptible to HEV infection [3]. These were previously archived pre-infection samples from blood donors who subsequently developed HEV viraemia and seroconverted to anti-HEV IgG.

Ethics

All immunodeficiency patients recruited provided written informed consent (National Health Service [NHS] Research Ethics Committee reference 04/Q0501/119). Testing of plasma from patients with acute and persistent HEV infection utilised archived residual samples that were sent to the national reference laboratory for routine diagnostics with consent for the remainder of samples to be used in

other assays. All experiments were performed in accordance with the 'Guidance on Conducting Research in Public Health England' (Version 3, October 2015; Document code RD001A).

Data collection

Patient demographics (age and sex), underlying diagnosis, immunoglobulin product infused and date of most recent infusion, iatrogenic immunosuppression and blood results (liver biochemistry and immunology) were obtained from patient notes, research databases and the laboratory pathology results system. Liver biochemistry results were available for 99% of patients within two months of sampling and lymphocyte subsets were available for 84% of patients within six months of sampling. For patients with low CD4 counts (<0.35 x 10^9/L), functional T-cell assays were reported when available.

Statistical Analysis

All statistics were performed in STATA/SE 13.1. Continuous data were compared using the Wilcoxon two-sample signed-rank test. Categorical data were compared using the Fisher's exact or Chi-squared test. Pre- and post-IVIG paired data were analysed using the Wilcoxon matched pairs test. Pearson's correlation was used to assess the relationship between time since immunoglobulin infusion and anti-HEV IgG level. Multivariable logistic regression was used to investigate the relationship between predictor variables and anti-HEV IgG seropositivity.

HEV RNA testing

Nucleic acid was extracted from 200µl of a primary sample on the MagnaPure 96 automated extraction platform (Roche Diagnostics Ltd. Burgess Hill, UK; virus-specific cell-free protocol). HEV

RNA was detected using 10µl of extract in an internally controlled and validated quantitative HEV PCR (expressed in international units per ml; IU/ml) as previously described (limit of detection 22 IU/ml) [20].

Anti-HEV IgG detection

Anti-HEV IgG was detected using the Wantai IgG assay in accordance with the manufacturer's recommendations (Fortress Diagnostics, Antrim, Northern Ireland, UK). Samples with a sample/cut-off ratio (S/CO) $\geq$1.1 were considered positive.

Each immunoglobulin product and the plasma samples from cases of HEV infection were ascribed a World Health Organisation (WHO) unitage (IU/ml). Briefly, the WHO HEV antibody standard (NIBSC code: 95/584) was reconstituted (assigned unitage of 100 units per ml) and tested in parallel dilutions to generate a sigmoid curve. Products and plasma samples were diluted in a pool of normal human plasma (NHP) and ascribed a unitage based on the mean S/CO values on the linear portion of the sigmoid curve. All NHP constituents were tested for and found non-reactive for anti-HEV IgG.

Quantification of HEV Antigen neutralisation

Immunoglobulin products and patient samples including nine samples pre- and post-IVIG, five plasma samples from acute HEV cases and five from previously persistent HEV cases recently recovered after ribavirin therapy were tested for the presence of HEV-Ag neutralising antibodies using a recently published method [21]. Prior to testing, samples from patients with acute and persistent HEV infection were assigned a WHO unitage for anti-HEV reactivity and then diluted in NHP to have an equivalent level of anti-HEV IgG of between two and five WHO IU/ml. In brief, HEV-Ag neutralising reactivity was measured by incubating 30 microlitres of diluted supernatant fluid from tissue culture expressing ORF2 HEV-Ag (day 45 post-inoculation of HepG2/C3a cell line with G3 HEV-containing faecal sample) with 30µl of test sample (immunoglobulin product, patient plasma or NHP control) for one hour at room temperature. Residual HEV-Ag reactivity was measured (HEV-Ag ELISA, Fortress Diagnostics, Antrim, Northern Ireland, UK)[22]. HEV-Ag neutralising activity was determined as a

percentage of reduction in reactivity in the HEV-Ag assay when the tissue culture supernatant was incubated with the test sample in comparison to incubation with a non-neutralising control (NHP):

$$\% \text{ neutralisation} = 100 - \frac{(OD_{\text{cultured antigen + test sample*}} - OD_{NHP})}{(OD_{\text{cultured antigen + NHP}} - OD_{NHP})} \times 100$$

OD = optical density at 450/630nm wavelength
NHP = normal human plasma (negative for HEV IgG)
* = immunoglobulin product or patient plasma

Results

Clinical Characteristics of the Study Population

The characteristics of the 245 patients tested for HEV RNA are presented in Table 1. The majority of patients (80.8%) had an underlying primary immunodeficiency syndrome and most (75.1%) were receiving intravenous immunoglobulin. Fifty seven patients were administered additional immunosuppressive medication (Table S1 supplementary material). Ninety patients had CD4+ T-cell counts below the normal range (<0.5 x 10^9/L) whilst 108 patients had CD19+ B-cell counts below the normal range (<0.10 x 10^9/L). Twenty three of 43 patients with significant CD4+ T-cell deficiency (<0.35 x 10^9/L) had T-cell proliferation results available, of which 74% displayed significantly impaired proliferation to phytohaemagglutinin, 68% to CD3 and 44% to CD3/CD28 stimulation. Thirty nine patients (16%) had an abnormal alanine aminotransferase (ALT) value at the time of testing for HEV infection.

HEV markers in antibody-deficiency patients

HEV RNA was not detected in any plasma sample from the 245 patients, confirming that none were viraemic. Anti-HEV IgG was detected (S/CO >1.1) in the plasma from 38.8% of patients. However, the anti-HEV reactivity even in those samples considered negative by the ELISA manufacturers criteria had a significantly different distribution than the reactivity of samples from HEV known-susceptible blood donors (Figure 1).

Factors associated with a patient being seropositive for anti-HEV IgG

Comparison of serological reactivity of patients' plasma revealed the type of immunoglobulin product administered was the only statistically significant factor predicting anti-HEV IgG seropositivity ($p<0.001$) (Table S2 supplementary material). Specifically the receipt of Kiovig 10%, Intratect 10% and Intratect 5% was significantly related to the detection of plasma anti-HEV IgG (S/CO >1.1).

Patients given Kiovig 10% and those given Intratect 5% were 24 times (95% CI 4.8-122.7) and 136 times (95% CI 2.5-7501) more likely to have detectable plasma anti-HEV IgG when compared to patients who received Flebogamma DIF 5% (Table 2). No relationship was seen between the time since immunoglobulin infusion (in days) and the level of anti-HEV IgG detected in the patient's plasma (Pearson's correlation -0.1763, data not shown), even when products were analysed individually. However, the variability of timings was not wide; most patients (77.4%) had received their last dose of immunoglobulin within 20-30 days of testing. Age, sex, underlying diagnosis, receipt of iatrogenic immunosuppression, route of immunoglobulin administration and patients' lymphocyte subset results demonstrated no relationship with anti-HEV IgG seropositivity.

All ten immunoglobulin products tested (8 IVIG and 2 SCIG) contained detectable anti-HEV IgG (range 0.12-7.40 WHO IU/ml) (Figure 2). There was some evidence of batch-to-batch variation in antibody titre, nevertheless all batches of Kiovig, Gamunex and Privigen were reactive for anti-HEV (Table S3 supplementary material). The likelihood of a patient being seropositive for anti-HEV IgG strongly correlated with the level of anti-HEV IgG in the product ($p < 0.001$, Figure 2).

HEV ORF2 antigen neutralising activity of patient plasma samples and immunoglobulin products

All ten immunoglobulin products were also able to neutralise HEV-Ag expressed in tissue culture and prevent reactivity in the HEV-Ag assay. Antigen neutralising activity was detectable at a high level in half of the products tested even at a dilution of 1:20 (Figure 3). The extent of antigen neutralisation correlated with the anti-HEV IgG S/CO value (Figure S1 supplementary material). In all nine patients, tested before and after infusion, the levels of anti-HEV IgG detected were higher in post-infusion samples (pre-infusion median S/CO 0.90; post-infusion median S/CO 1.96, p=0.008) and correlated with higher antigen neutralising activity in eight of the nine patients (p=0.015) (Table 3).

The source of anti-HEV affects the antigen neutralising capacity

Five plasma samples from acute HEV cases and five from persistent HEV cases recently recovered after ribavirin therapy were tested for anti-HEV IgG in a half-log dilution series alongside the WHO antibody reference. A dilution of each sample containing equivalent levels of anti-HEV IgG (between 2 and 5 WHO IU/ml) was then tested for presence of HEV-Ag neutralising antibody. The antigen neutralising capability was significantly higher in the dilutions of the plasma samples from healthy patients recovered recently from acute HEV infection (median 81.5%, range 75.7-93.2%) compared with that seen in plasma from patients recovered from persistent HEV infection (median 31.1%, range 25.4-45.8%) (p=0.009) (Figure 4). A direct comparison of antigen neutralising activity at the equivalent level of anti-HEV IgG in plasma from patients post-IVIG was not possible due to much lower anti-HEV in the latter (median S/CO 1.96, equivalent to ~0.2 WHO IU/ml). However, antigen neutralising activity was broadly similar between post-IVIG patient samples (median 91.0%, range 74.2-99.7%) and plasma from acute cases (median 81.5%, range 75.7-93.2%) despite much lower anti-HEV IgG detected in the same materials by ELISA.

Discussion

We found no persistent HEV infections in a cohort of 245 patients with primary or secondary antibody deficiency, despite evidence of significant immunocompromise with low CD19+ B-cell counts (45% <0.10 x 10^9/L) and CD4+ T-cell counts (37% <0.5 x 10^9/L). Nearly a quarter were also taking immunosuppressive medication. This is a significant finding in the current context of a high risk of HEV acquisition from dietary sources in the UK and the longstanding immune dysfunction in patients with antibody deficiency which renders them susceptible to a number of persistent and severe viral infections [2, 14-17, 23-25]. The results are consistent with studies in similar cohorts, neither of which displayed any evidence of persistent HEV infection amongst 73 CVID patients in Germany or 27 primary antibody deficient patients with deranged liver enzymes in the UK [13, 18]. Our study, which was larger and more heterogeneous, adds to the growing body of evidence that there is an extremely low risk of persistent HEV infection in these groups of patients. However, even larger studies may be required to detect a very small risk of HEV infection given that the prevalence of HEV viraemia in cohorts considered high risk for persistent HEV infection such as solid organ transplant recipients in Western Europe is low (0.7-1.5%) [26-30]. We tested for HEV RNA regardless of liver enzyme values because of the uncertainty of whether an abnormal ALT would be a sufficiently sensitive predictor of active HEV infection; at the time of HEV testing 16% of patients had abnormal ALT values.

The reason for the absence of persistent HEV infection in these patients could be due to a number of factors. We detected anti-HEV IgG in a high proportion of patient's plasma in this study (38.8%). Many plasma samples testing seronegative by the ELISA manufacturers' criteria nevertheless had higher anti-HEV IgG optical density values compared with plasma from blood donors known to be susceptible to HEV infection. This suggests the presence of low levels of anti-HEV IgG in the patient samples but where the ELISA reactivity falls below the manufacturers' defined cut-off. Detectable anti-HEV IgG in plasma of antibody deficient patients could be the result of passive acquisition of antibodies from the immunoglobulin products, residual endogenous antibody production or a combination of the two. The most compelling explanation is passive acquisition of anti-HEV, supported by the correlation of patient plasma reactivity for anti-HEV IgG with both receipt of certain immunoglobulin products and the titre of anti-HEV IgG in those products. Rising titres of anti-HEV IgG observed post-IVIG in patients tested before and after infusion reinforce this assertion.

In this study we also demonstrate that patients' plasma and immunoglobulin products were able to bind to HEV ORF2 Ag and prevent or reduce reactivity in the HEV-Ag assay, referred to as antigen neutralisation. This antigen neutralising activity also rose in post-IVIG infusion samples in the subset of patients tested concordantly with rises in anti-HEV IgG levels. Recent studies exploring different forms of ORF2 antigen suggest that the antigen neutralisation we are detecting may be directed predominantly against the abundant secreted form of ORF2 which is not virion-associated [31, 32]. Nevertheless, this secreted form of ORF2 is still considered to harbour the major neutralizing epitopes found on the capsid form of ORF2 [32, 33]. We postulate that the presence of pre-existing antigen neutralising antibodies circulating in their plasma may be sufficient to protect these patients from an enteric challenge of HEV and prevent early infection or the establishment of persistent infection.

It is notable that we found that the source of anti-HEV IgG influences the antigen neutralising capacity. It is well documented that patients with persistent infection themselves produce anti-HEV antibodies without eliminating the virus, therefore we tested plasma from five acute HEV cases and five recovered persistent HEV cases for the ability of the detectable anti-HEV antibody to neutralise the tissue culture-derived antigen [6]. We demonstrate that the antibodies from recovered persistent HEV cases had significantly lower antigen neutralising capacity when compared with acute HEV cases when tested at equivalent levels of anti-HEV IgG (between 2 and 5 WHO IU/ml). Due to lower anti-HEV IgG titres in the plasma from patients following IVIG infusions (7/9 samples had <1 WHO IU/ml) we could not perform a direct comparison at an equivalent WHO unitage; despite this, the antigen neutralising activity was similar in post-IVIG samples compared to plasma from recovered acute cases. This higher antigen neutralising activity relative to the detected anti-HEV IgG titre may explain why these patients either do not develop initial HEV infection or do not develop persistence despite comparatively low concentrations of anti-HEV detected by ELISA.

We are unable to conclude definitively that passively transferred HEV antibodies are protective for our patients, since correlates of protection against HEV infection are undefined even in vaccine studies [34]. However, as proof-of-concept, late stage convalescent plasma has been used successfully to prevent cynomolgus monkeys from developing hepatitis after an HEV challenge, therefore IVIG may prove useful for prevention of HEV infection [35]. Indeed IVIG has shown promise as a therapeutic agent in a small number of cases of HEV-associated neuralgic amytrophy [36]. The recent description

of a model for HEV infection using humanized homozygous uPA+/+-SCID mice may enable passive immunoprophylaxis to be studied further [37]. An alternative explanation for the lack of persistent HEV infections in this study is that enough of the patients had sufficient preserved T-cell activity to clear HEV following infection. Suneetha *et al* have demonstrated the importance of T-cell responses for control of HEV infection [38]. The initial description of persistent HEV infections in solid organ transplant recipients found significantly lower total lymphocytes, CD2+, CD3+ and CD4+ cell counts in individuals developing persistent infection compared to those who resolved HEV infection [6]. In our study 77% of patients had normal levels of CD8+ T-cells (>0.2 x 10^9/L) and higher median total lymphocyte count, CD3+ and CD4+ cell counts than found by Kamar *et al* in persistently infected transplant patients [6]. However, T-cell deficiency and iatrogenic immunosuppression were not uncommon in our patients and even in this subset no viraemic cases were detected.

In summary, we found no evidence of persistent HEV infection in an immunocompromised cohort of patients with primary and secondary antibody deficiency. The HEV-Ag neutralising antibodies detected in both immunoglobulin products and patients' plasma may provide sufficient protection from developing HEV infection. Nevertheless we agree with Mohamed *et al* that patients in these groups with persistently elevated liver enzymes should still be tested for HEV RNA [13]. Further work on elucidating the relative importance of T-cell and B-cell responses for HEV clearance are needed to help stratify risk in these and other immunocompromised cohorts.

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References

1. Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E. The Lancet **2012**; 379:2477-88.

2. Adlhoch C, Avellon A, Baylis SA, et al. Hepatitis E virus: Assessment of the epidemiological situation in humans in Europe, 2014/15. J Clin Virol **2016**; 82:9-16.

3. Hewitt PE, Ijaz S, Brailsford SR, et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet **2014**; 384:1766-73.

4. Schlosser B, Stein A, Neuhaus R, et al. Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient. J Hepatol **2012**; 56:500-2.

5. Pourbaix A, Ouali N, Soussan P, et al. Evidence of hepatitis E virus transmission by renal graft. Transpl Infect Dis 2017 Feb;19(1) doi: 101111/tid12624 Epub 2017 Jan 10 **2017**.

6. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N Engl J Med **2008**; 358:811-7.

7. Kenfak-Foguena A, Schoni-Affolter F, Burgisser P, et al. Hepatitis E Virus seroprevalence and chronic infections in patients with HIV, Switzerland. Emerg Infect Dis **2011**; 17:1074-8.

8. Fraticelli P, Bagnarelli P, Tarantino G, et al. Chronic hepatitis E in a patient treated with rituximab and mycophenolate mofetil for Sjogren's syndrome. Rheumatology (Oxford) **2016**; 55:2275-7.

9. Tavitian S, Peron JM, Huynh A, et al. Hepatitis E virus excretion can be prolonged in patients with hematological malignancies. J Clin Virol **2010**; 49:141-4.

10. Ollier L, Tieulie N, Sanderson F, et al. Chronic hepatitis after hepatitis E virus infection in a patient with non-Hodgkin lymphoma taking rituximab. Ann Intern Med **2009**; 150:430-1.

11. van Dijk WEM, Vergeer M, Arends JE. Persistent Hepatitis E Infection in a Patient with Tuberous Sclerosis Complex Treated with Everolimus: A Case Report. Infect Dis Ther **2017**; 6:291-5.

12. Bettinger D, Schorb E, Huzly D, et al. Chronic hepatitis E virus infection following allogeneic hematopoietic stem cell transplantation: an important differential diagnosis for graft versus host disease. Ann Hematol **2015**; 94:359-60.

13. Mohamed OE, Jones J, Osman H, Huissoon AP. Unexplained abnormal liver function in patients with primary antibody deficiency: could it be chronic hepatitis E infection? J Clin Pathol 2017 Aug 9 doi: 101136/jclinpath-2017-204627 **2017**.

14. Bjoro K, Froland SS, Yun Z, Samdal HH, Haaland T. Hepatitis C infection in patients with primary hypogammaglobulinemia after treatment with contaminated immune globulin. N Engl J Med **1994**; 331:1607-11.

15. Brown LK, Clark I, Brown JR, Breuer J, Lowe DM. Norovirus infection in primary immune deficiency. Rev Med Virol 2017 Mar 8 doi: 101002/rmv1926 **2017**.

16. Kainulainen L, Vuorinen T, Rantakokko-Jalava K, Osterback R, Ruuskanen O. Recurrent and persistent respiratory tract viral infections in patients with primary hypogammaglobulinemia. J Allergy Clin Immunol **2010**; 126:120-6.

17. Bearden D, Collett M, Quan PL, Costa-Carvalho BT, Sullivan KE. Enteroviruses in X-Linked Agammaglobulinemia: Update on Epidemiology and Therapy. J Allergy Clin Immunol Pract **2016**; 4:1059-65.

18. Pischke S, Horn-Wichmann R, Ernst D, et al. Absence of chronic hepatitis E in a German cohort of common variable immunodeficiency patients. Infect Dis Rep **2012**; 4:e28.

19. Ramsay I, Gorton RL, Patel M, et al. Transmission of Hepatitis B Core Antibody and Galactomannan Enzyme Immunoassay Positivity via Immunoglobulin Products: A Comprehensive Analysis. Clin Infect Dis **2016**; 63:57-63.

20. Garson JA, Ferns RB, Grant PR, et al. Minor groove binder modification of widely used TaqMan probe for hepatitis E virus reduces risk of false negative real-time PCR results. J Virol Methods **2012**; 186:157-60.

21. Ankcorn MJ, Ijaz S, Haywood B, et al. Confirmation of specificity of reactivity in a solid phase ELISA for the detection of hepatitis E viral antigen improves utility of the assay. Journal of Virological Methods **2018**; 252:42-8.

22. Tanaka T, Takahashi M, Kusano E, Okamoto H. Development and evaluation of an efficient cell-culture system for Hepatitis E virus. J Gen Virol **2007**; 88:903-11.

23. Tedder RS, Ijaz S, Kitchen A, et al. Hepatitis E risks: pigs or blood-that is the question. Transfusion **2017**; 57:267-72.

24. Duraisingham SS, Buckland M, Dempster J, Lorenzo L, Grigoriadou S, Longhurst HJ. Primary vs. secondary antibody deficiency: clinical features and infection outcomes of immunoglobulin replacement. PLoS One **2014**; 9:e100324.

25. Ruffner MA, Sullivan KE, Henrickson SE. Recurrent and Sustained Viral Infections in Primary Immunodeficiencies. Front Immunol **2017**; 8:665.

26. Pas SD, de Man RA, Mulders C, et al. Hepatitis E virus infection among solid organ transplant recipients, the Netherlands. Emerg Infect Dis **2012**; 18:869-72.

27. Pischke S, Stiefel P, Franz B, et al. Chronic hepatitis e in heart transplant recipients. Am J Transplant **2012**; 12:3128-33.

28. Haagsma EB, Niesters HG, van den Berg AP, et al. Prevalence of hepatitis E virus infection in liver transplant recipients. Liver Transpl **2009**; 15:1225-8.

29. Pischke S, Greer M, Hardtke S, et al. Course and treatment of chronic hepatitis E virus infection in lung transplant recipients. Transpl Infect Dis **2014**; 16:333-9.

30. Buffaz C, Scholtes C, Dron AG, et al. Hepatitis e in liver transplant recipients in the Rhone-Alpes region in France. European Journal of Clinical Microbiology and Infectious Diseases **2014**; 33:1037-43.

31. Montpellier C, Wychowski C, Sayed IM, et al. Hepatitis E Virus Lifecycle and Identification of 3 Forms of the ORF2 Capsid Protein. Gastroenterology **2018**; 154:211-23 e8.

32. Yin X, Ying D, Lhomme S, et al. Origin, antigenicity, and function of a secreted form of ORF2 in hepatitis E virus infection. Proc Natl Acad Sci U S A **2018**.

33. Zhang J, Gu Y, Ge SX, et al. Analysis of hepatitis E virus neutralization sites using monoclonal antibodies directed against a virus capsid protein. Vaccine **2005**; 23:2881-92.

34. Zhao Q, Zhang J, Wu T, et al. Antigenic determinants of hepatitis E virus and vaccine-induced immunogenicity and efficacy. J Gastroenterol **2013**; 48:159-68.

35. Tsarev SA, Tsareva TS, Emerson SU, et al. Successful passive and active immunization of cynomolgus monkeys against hepatitis E. Proc Natl Acad Sci U S A **1994**; 91:10198-202.

36. van Eijk JJJ, Dalton HR, Ripellino P, et al. Clinical phenotype and outcome of hepatitis E virus-associated neuralgic amyotrophy. Neurology **2017**; 89:909-17.

37. Sayed IM, Verhoye L, Cocquerel L, et al. Study of hepatitis E virus infection of genotype 1 and 3 in mice with humanised liver. Gut **2017**; 66:920-9.

38. Suneetha PV, Pischke S, Schlaphoff V, et al. Hepatitis E virus (HEV)-specific T-cell responses are associated with control of HEV infection. Hepatology **2012**; 55:695-708.

Table 1. Characteristics of antibody-deficient patients tested for HEV RNA.

Characteristic		No. (%)
Sex		
Male		91 (37.1)
Female		154 (62.9)
Age, yrs, Median [IQR]		56 [41-68]
Underlying Diagnosis		
CVID		138 (56.3)
XLA		15 (6.1)
Hyper IgM syndrome		3 (1.2)
Good's syndrome		1 (0.4)
Other primary antibody deficiency[a]		41 (16.7)
Secondary antibody deficiency[b]		47 (19.2)
Immunoglobulin Product/manufacturer		
IV	Flebogamma DIF 5%/Grifols	39 (15.9)
	Gammagard 10%/Shire	1 (0.4)
	Gammaplex 10%/BPL	20 (8.2)
	Gamunex 10%/Grifols	13 (5.3)
	Intratect 10%/Biotest	11 (4.5)
	Intratect 5%/Biotest	6 (2.5)
	Kiovig 10%/Baxalta	26 (10.6)
	Octagam 10%/Octapharma	15 (6.1)
	Privigen 10%/CSL Behring	53 (21.6)
SC	Gammanorm 16.5%/Octapharma	10 (4.1)
	Hizentra 20%/CSL Behring	24 (9.8)

Subcuvia 16%/Baxalta	13 (5.3)
Subgam 16%/BPL	14 (5.7)
Iatrogenic immunosuppression	
Nil	188 (76.7)
Mono or combination therapy	57 (23.3)
Lymphocyte count, x 10^9/L, Median [IQR]	1.39 [1.01-1.89][c]
No. (%) < normal range (1.0)	58 (24.3)[c]
CLL patients only (n=6), Median [IQR]	3.20 [1.55-13.73]
Absolute CD3 count[d], x 10^9/L, Median [IQR]	1.08 [0.74-1.49]
No. (%) < normal range (0.7)	54 (22.0)
Absolute CD4 count[d], x 10^9/L, Median [IQR]	0.67 [0.41-0.83]
No. (%) <0.5	90 (36.7)
No. (%) <0.35	43 (17.6)
Absolute CD8 count[d], x 10^9/L, Median [IQR]	0.35 [0.21-0.62]
No. (%) < normal range (0.2)	56 (22.9)
Absolute CD19 count, x 10^9/L, Median [IQR]	0.11 [0.03-0.23][d]
No. (%) < normal range (0.10)	108 (45.2)[d]
CLL patients only (n=6), Median [IQR]	0.71 [0.42-11.95]
Absolute CD16+CD56[d], x 10^9/L, Median [IQR]	0.13 [0.07-0.21]
No. (%) < normal range (0.09)	78 (31.8)
ALT[d], IU/L, Median [IQR]	24 [19-33]
No. (%) > normal range[e]	39 (16.0)
Bilirubin[d], umol/L, Median [IQR]	6 [5-9]
No. (%) > normal range (21)	5 (2.0)

[a]Includes hypogammaglobulinaemia, IgG subclass deficiency and specific antibody deficiency. isolated IgG

[b]Lymphoma +/- rituximab (n=15), chronic lymphocytic leukaemia (n=6), multiple myeloma or monoclonal gammopathy of uncertain significance (n=7), allograft HSCT (n=3), rheumatoid or vasculitis+/- rituximab (n=10), medication induced (n=6).

[c]Excludes CLL patients (n=6) and missing data for 2 patients (2 further patients missing CD19+ counts).

[d]Missing data for 2 patients.

[e]ULN of ALT varied by year sampled and by gender (women: > 33IU/L in 2015, >35 in 2016/17, men: >41 in 2015, >50 in 2016/17.)

Abbreviations: ALT, alanine aminotransferase; CLL, chronic lymphocytic leukaemia; CVID, common variable immune deficiency; IQR, interquartile range; XLA, X-linked agammaglobulinaemia.

Table 2. Multivariable model examining the relationship between product type administered and a patient testing anti-HEV IgG reactive

IVIG Product	Odds Ratio[a]	P value[a]	95% CI[a]
Flebogamma DIF 5%	1.00		
Gammaplex 10%	0.52	0.588	0.05-5.58
Gamunex 10%	1.59	0.644	0.22-11.24
Intratect 10%	incalculable[b]	-	-
Intratect 5%	135.92	0.016	2.46-7500.68
Kiovig 10%	24.21	<0.001	4.78-122.69
Octagam 10%	4.49	0.076	0.85-23.59
Privigen 10%	1.71	0.504	0.36-8.13

Data only considered if time since infusion was available (n=133). No data was available for Gammagard 10%, Gammanorm 16.5%, Hizentra 20%, Subcuvia 16% or Subgam 16%.

[a]Multivariable logistic regression was used to investigate the relationship between predictor variables and anti-HEV IgG seropositivity. The final model adjusted for time since infusion (days).

[b]All patients on Intratect 10% tested anti-HEV IgG positive therefore an odds ratio could not be calculated.

Abbreviations: CI, confidence interval; IVIG, intravenous immunoglobulin.

Table 3. Pre and Post IVIG anti-HEV IgG levels and neutralisation activity in nine patients.

Patient no.	IVIG Product infused	Pre-IVIG		Post-IVIG	
		anti-HEV IgG S/CO	% Neutralisation	anti-HEV IgG S/CO	% Neutralisation
1	Privigen 10%	1.27	82.29	2.35	90.97
2	Intratect 10%	11.62	99.13	14.15	99.65
3	Privigen 10%	6.10	98.44	7.60	97.57
4	Kiovig 10%	0.36	36.98	3.02	97.92
5	Flebogamma DIF 5%	0.41	57.64	1.77	90.46
6	Privigen 10%	0.90	73.63	1.39	87.38
7	Gammaplex 10%	0.28	42.78	0.81	74.19
8	Gamunex 10%	0.76	47.27	1.96	95.23
9	Privigen 10%	1.05	74.76	1.75	81.49

Nine patients were tested for anti-HEV IgG and the presence of HEV-Ag neutralising antibodies prior to IVIG infusion and immediately post infusion. Anti-HEV IgG rose in all nine patients (median OD change 0.2 IQR [0.1-0.3], p=0.008) and neutralising activity rose post infusion in 8/9 patients (median change in % of neutralisation 13.7 IQR [6.7-32.8], p=0.015) (Wilcoxon signed-rank test). The percentage of neutralisation was calculated as a percentage of the reduction in binding (see methods).

Abbreviations: HEV-Ag, hepatitis E virus antigen; IVIG, intravenous immunoglobulin.

Figure 1. Frequency density plot of anti-HEV IgG reactivity in antibody-deficient patients on immunoglobulin replacement therapy compared with HEV-uninfected blood donors. The lines represent fitted distributions which were a single normal distribution for blood donors and a mix of two normal distributions for the antibody deficient patients. Blood donor samples were archived plasma samples prior to the development of HEV infection to represent true anti-HEV IgG negative samples. There was a significant difference between the median S/CO values in HEV-uninfected blood donors (median, 0.06) and the antibody-deficient patients (median, 0.68) ($p<0.001$). Data was transformed to a log-scale for analysis; the axis labels were back-transformed for presentation. The hatched vertical line represents the manufacturer's cut-off (S/CO 1.1) for positive results.

Abbreviations: ELISA, enzyme linked immunosorbent assay; S/CO, sample over cut-off of optical density.

Figure 2. Correlation of the percentage of patients on each product testing anti-HEV IgG reactive and the IgG level (WHO IU/ml) detected in the product. Ten immunoglobulin products were tested in duplicate for anti-HEV IgG. The ascribed level of WHO IU/ml was plotted against the proportion of patients on each product testing anti-HEV IgG reactive (S/CO >1.1) and showed a good correlation (Pearson's correlation 0.900, $p = 0.0004$). The lowest result for any product was still reactive for anti-HEV IgG by manufacturer's criteria (mean S/CO of 2.44 for Gammaplex 10% equivalent to 0.12 WHO IU/ml).

Abbreviations: IV, intravenous. SC, subcutaneous. IU, international units.

Figure 3. HEV-Ag neutralising activity of immunoglobulin products at differing dilutions. Ten different immunoglobulin products were titrated in normal human plasma up to 1:320 and tested for the presence of antibodies capable of neutralising HEV-Ag derived from cell culture. The percentage of neutralisation was calculated as a percentage of the reduction in binding (see methods).
Abbreviations: HEV-Ag, hepatitis E virus antigen. SC, subcutaneous.

Figure 4. Comparison of HEV-Ag neutralisation by plasma from acute clinical cases of HEV, recovered persistent cases of HEV and patients post-IVIG. The neutralising activity of plasma samples diluted to have an equivalent level of anti-HEV IgG (between 2 and 5 WHO IU/ml) from (A) patients recently recovered from persistent HEV infection (median 31.1%, range 25.4-45.8%) was significantly lower than plasma samples from (B) patients in recovery from acute HEV infection (median 81.5%, range 75.7-93.2%) (p=0.009). Plasma samples from (C) post-IVIG patients were tested undiluted as they had lower anti-HEV IgG (median 0.22 WHO IU/ml, range 0.05-15.9) and therefore could not be diluted to comparable level of anti-HEV IgG, but still exhibited high neutralising activity similar to acute HEV cases (median 91.0%, range 74.2-99.7%).

Abbreviations: HEV, hepatitis E virus; IVIG, intravenous immunoglobulin; HEV-Ag, hepatitis E virus antigen.

Figure 1.

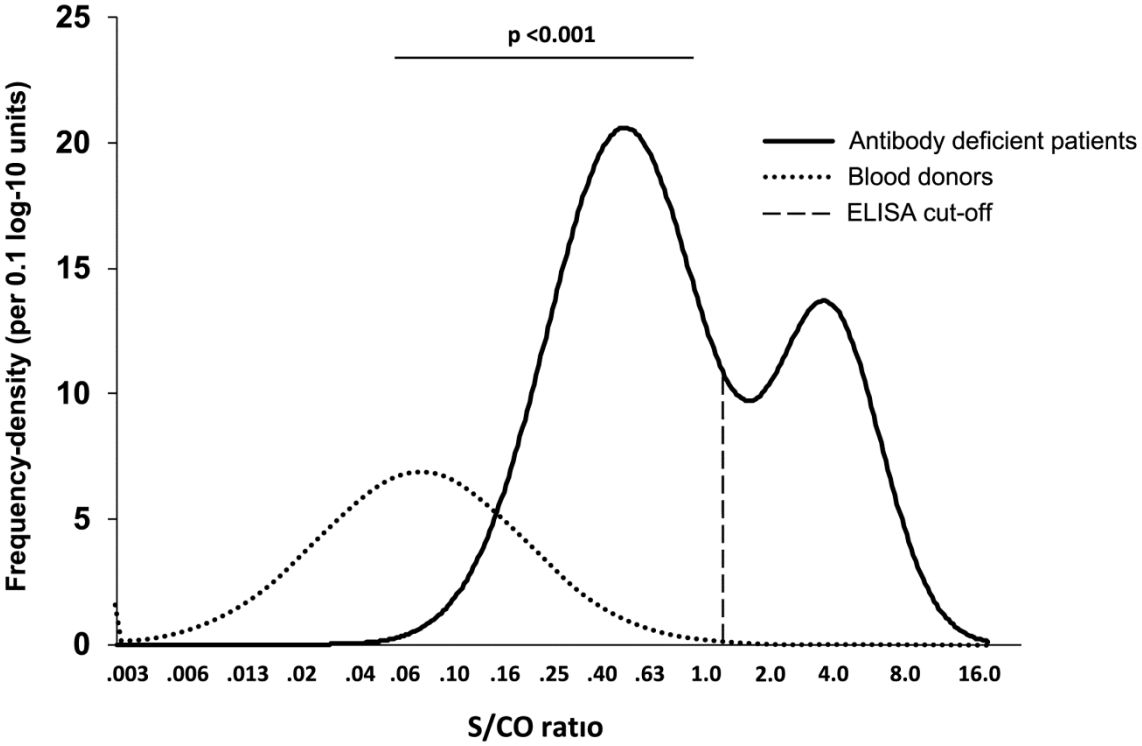

Figure 2.

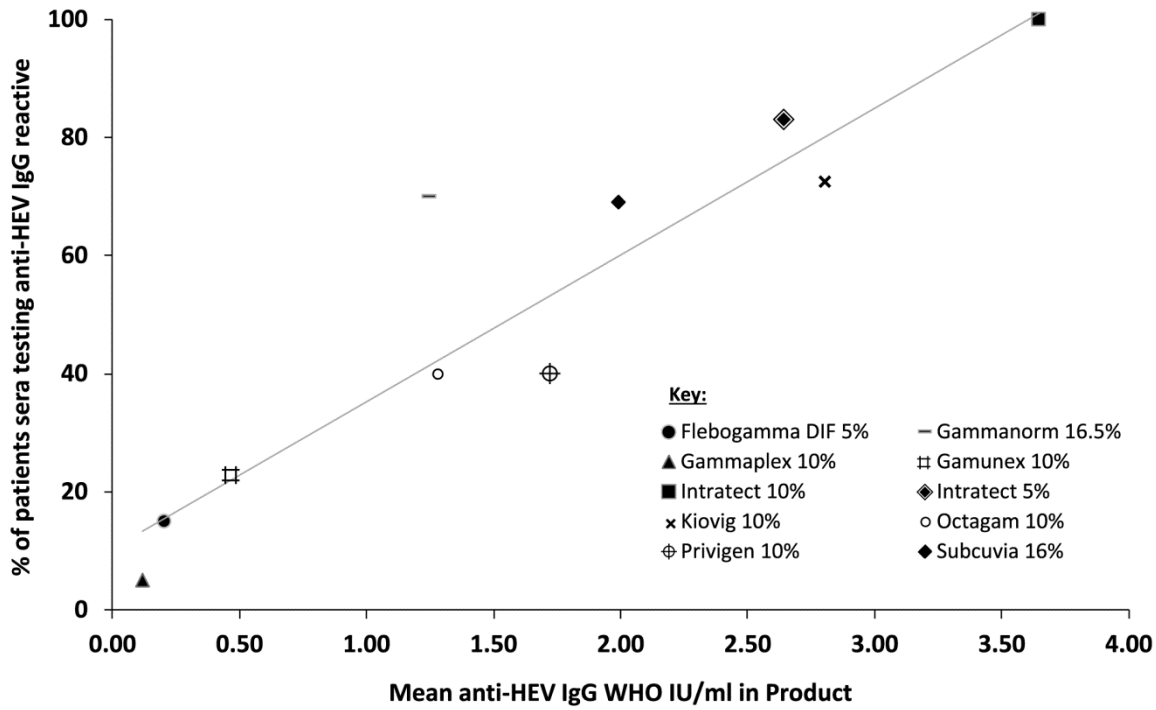

Figure 3.

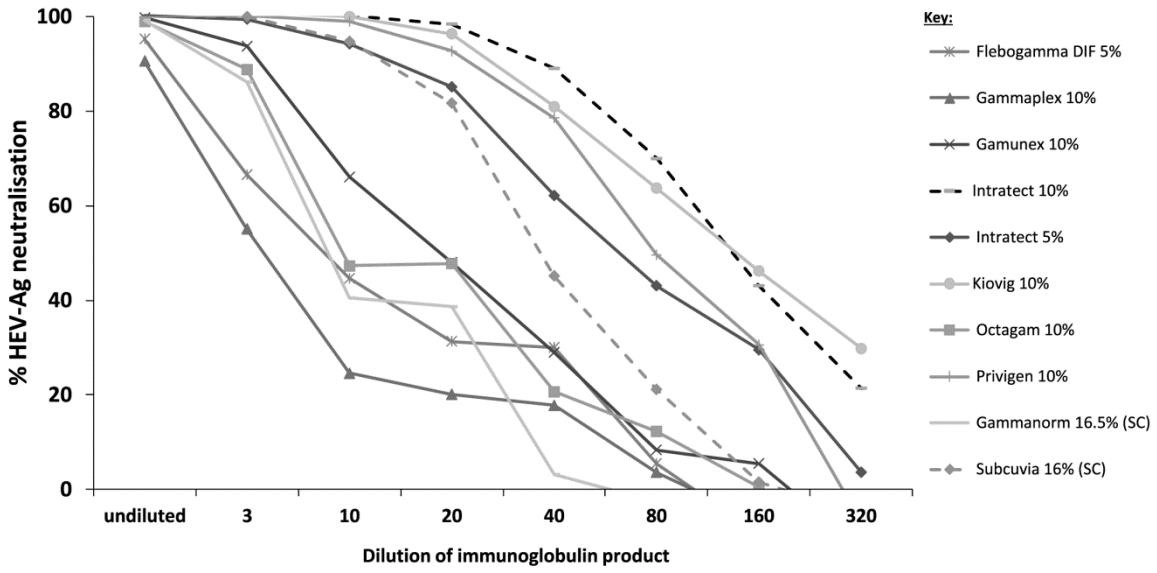

Figure 4.