Characterising the risk of HEV infection in Haematological Malignancies: a UK prospective prevalence study

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Abbreviations

ALT, alanine aminotransferase

CI, Confidence interval

DILI, Drug-induced liver injury

G3, Genotype 3

GvHD, Graft-versus-host-disease

HEV, Hepatitis E virus

HEV-Ag, Hepatitis E virus antigen

HSCT, Haematopoietic stem cell transplant

IU, international units

IQR, interquartile range

MDS, myelodysplastic syndrome

MPN, myeloproliferative neoplasm

ND, Not detected

NHS, National Health Service

NHSBT, National Health Service Blood and Transplant

ORF2, Open reading frame 2

PICS, Prescribing Information & Communication System

PHE, Public Health England

PPV, Positive predictive value

RNA, ribonucleic acid

SaBTO, The Advisory Committee on the Safety of Blood, Tissues and Organs (UK)

SOT, Solid organ transplant.

ULN, Upper limit of normal

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Summary

Persistent hepatitis E virus (HEV) infection affects immunocompromised patients leading to cirrhosis in a proportion. The risk of persistent HEV in patients with haematological malignancies without allogeneic stem cell transplant is unknown. We established a single-centre prospective prevalence study of HEV viraemia in 1591 patients with haematological malignancies under active follow-up to inform testing strategies. In addition to HEV RNA testing, all samples also underwent testing for anti-HEV IgG and HEV antigen (HEV-Ag). Two of 1591 patients were viraemic with genotype 3 HEV giving a prevalence of 0.13% (95% CI, 0.02-0.45%). Both viraemic patients were being treated for progressive multiple myeloma and developed persistent HEV infection. Seropositivity for anti-HEV IgG was 16.7% which rose with increasing age and the number of blood transfusions received. The HEV-Ag assay was highly specific (99.5%) but failed to detect the earliest sample in one of the two viraemic patients. The very low rate of active HEV infection in this heterogeneous cohort of patients under follow up for haematological malignancy do not support the need for routine unselected screening of patients. Increasing transfusion exposure was associated with an increased risk of HEV acquisition as ascertained by seropositivity for anti-HEV IgG.

Word count 195

Keywords: Hepatitis E virus, Multiple Myeloma, screening, chronic hepatitis e virus infection, immunocompromised patients.

Introduction

Hepatitis E virus (HEV) is a common infection worldwide. There are four major genotypes that affect humans; genotypes 1 and 2 (G1 and G2) are faeco-orally transmitted in areas of poor sanitation whereas genotype 3 and 4 (G3 and G4) are acquired as a foodborne zoonosis. G3 is found worldwide in swine, deer and rabbits and is the only genotype circulating in animals in the UK known to infect humans (Pavio*, et al* 2015). It is transmitted predominantly through consumption of pork products but also through substances of human origin including blood transfusions and organ transplantation (Hewitt*, et al* 2014, Pourbaix*, et al* 2017, Schlosser*, et al* 2012). In recent years there is evidence of increased risk of HEV acquisition reflected in rising clinical cases and a rising prevalence of viraemia in blood donors (1:2800) (Adlhoch*, et al* 2016, Hewitt*, et al* 2014).

Persistent infections were first described in solid organ transplant (SOT) recipients and have since been reported in auto-immune disease, HIV-infected patients and haematopoietic stem cell transplant (HSCT) recipients (Bettinger*, et al* 2015, Debes*, et al* 2016, Kamar*, et al* 2008a, Kamar*, et al* 2008b, Koenecke*, et al* 2012, van der Eijk*, et al* 2014, Versluis*, et al* 2013). The prevalence of infection in these patient groups varies geographically and by patient group. In SOT recipients in Western Europe HEV viraemia prevalence varies between 0.5 and 3.2% (Ankcorn*, et al* 2018b, Legrand-Abravanel*, et al* 2011, Pas*, et al* 2012, Pischke*, et al* 2014, Reekie*, et al* 2018). Small studies in HSCT recipients have found rates of HEV viraemia between 0.4% in the peri-transplant period to as high as 2.4% (Ankcorn*, et al* 2018b, Reekie*, et al* 2018, Versluis*, et al* 2013).

Around 60% of SOT recipients acquiring HEV will develop a persistent infection and of these, 10% will develop liver cirrhosis (Kamar*, et al* 2014, Kamar*, et al* 2008b). In patients with underlying haematological malignancy the natural history is less well defined. A number of case reports describe persistent courses of HEV infection including fatal outcomes in patients with haematological malignancies outside the context of allogeneic-HSCT (Alnuaimi*, et al*

2017, Gauss*, et al* 2012, Ollier*, et al* 2009, Pfefferle*, et al* 2012). In one study five of 14 (36%) patients with haematological malignancies acquiring acute HEV infection developed a persistent infection (Tavitian*, et al* 2015). Importantly the finding of active HEV infection in such patients can have important implications for their treatment schedule as the majority of therapeutic interventions for haematological malignancies result in a degree of immunosuppression. In patients undergoing treatment for a haematological malignancy who are found to have concomitant HEV infection, there is a need to strike a careful balance between increased immunosuppression for the haematological disorder and the detrimental effect this may have on the HEV infection; in many cases antiviral treatment may be required (Tavitian*, et al* 2015, Versluis*, et al* 2013). However, the magnitude of risk of persistent HEV in patients with haematological malignancies is unknown, with no systematic studies in patients who have not been treated with allogeneic-HSCT (Pischke*, et al* 2014, Pischke*, et al* 2012).

Patients with haematological malignancies are a heterogeneous group treated with increasingly diverse therapeutic regimens that cause varying degrees of immunosuppression. In addition the excess blood transfusion requirements in this patient group leads to increased exposure to HEV from donors (Hewitt*, et al* 2014). In the UK this risk from blood has been mitigated significantly by the implementation of donation screening by HEV RNA testing of pooled donations, initially through selective screening and later in April 2017 by universal screening (Hewitt*, et al* 2014, Tedder*, et al* 2017).

We set out to determine the point prevalence of HEV viraemia in an unselected cohort of patients with haematological malignancies to assess the need for routine screening using anti-HEV IgG seroprevalence as a marker of HEV risk. The utility of HEV-Ag detection as a screening assay in this cohort was also assessed.

Methods

Study Design

A single-centre prospective prevalence study of HEV viraemia in patients with haematological malignancies. Patients were tested if they had a diagnosis of haematological malignancy under active follow up; allograft HSCT recipients were excluded as a screening strategy was already in place for these patients.

In addition to HEV RNA testing, all samples also underwent testing for anti-HEV IgG and HEV-Ag.

Patient samples and data collection

Between 27th March and 1st September 2017, 1591 patients were identified at a large tertiary haematology referral centre in London from dedicated haematology inpatient wards and outpatient clinics (Lymphoma, Myeloma, CLL and MPN).

An extra EDTA sample was taken from each patient; plasma was spun, separated and stored at -20°C locally prior to shipping once weekly to the Blood Borne Virus Unit, Virus Reference Department, Public Health England for testing. A clinical report was provided for each sample tested.

Patient demographics, underlying primary haematological disease, disease status (classified as no remission, partial remission, complete remission, progressive disease), lines of treatment including the use of small molecule inhibitors or monoclonal antibodies, specific immunosuppressive medication in the preceding six months and blood results (total white cell count (WCC), lymphocyte count, neutrophil count, alanine aminotransferase (ALT) values and bilirubin values) at the time of HEV RNA testing were collected from patient records.

The numbers of transfused blood components given to each patient in the preceding five years before enrolment at centre was collected directly from the blood transfusion laboratory information management system (Bank Manager, Sussex Biologicals, UK). Transfusions were only considered in the five years prior to HEV RNA testing for each individual patient and any transfusions given after 10th April 2017 were excluded as this was the implementation date of

universal screening of blood donors in England for HEV RNA. Prior to universal screening this cohort were not given HEV-screened blood within our centre.

Study approval and management of HEV-infected patients

The Haematology department at UCLH approved the study as a survey to assess the prevalence of HEV viraemia to determine the need to extend HEV screening to this cohort. Routine screening for HEV was already in place for recipients of allogeneic HSCT, therefore the study was considered as an assessment of the need to extend screening to the wider haematological cohort.

Patients were informed of HEV testing by patient information leaflets and were given the choice of opting out of the testing service. Telephone or direct face-to-face support by a clinical nurse specialist was offered to any patient with any further specific queries about the study. All HEV RNA results were communicated via standard laboratory resulting platforms.

Abnormal results were emailed to the lead consultant of the patient; HEV viraemic patients were informed of the result by the clinical team, a confirmatory test undertaken and the patient reviewed by the Hepatologist in accordance with standard clinical practice.

HEV RNA testing

Nucleic acid was extracted on the MagNA Pure 96 (Roche Diagnostics Ltd. Burgess Hill, UK; virus-specific cell-free protocol) from 200µl of plasma spiked with 20µl of MS2 bacteriophage internal control. HEV RNA was detected and quantified from 10ul of extract using an in-house validated quantitative HEV PCR (expressed in international units per ml; IU/ml) as previously described (limit of detection 22 IU/ml) (Garson*, et al* 2012). Samples harbouring HEV RNA underwent sequence and phylogenetic analysis across part of the open reading frame 2 (ORF2) of HEV as previously described to assign a genotype and subtype (Ijaz*, et al* 2005).

HEV serology

All samples underwent anti-HEV IgG testing using the Wantai IgG detection assay in accordance with manufacturer's recommendations (Fortress Diagnostics, Antrim, Northern Ireland, UK). Patients were considered anti-HEV IgG seroreactive if the sample/cut-off (S/CO) ratio was $\geq$ 1.1. HEV RNA-positive samples were also tested for anti-HEV IgM using the Wantai IgM assay (Fortress Diagnostics, Antrim, Northern Ireland, UK).

HEV antigen testing and confirmation of reactivity

All samples were tested for the presence of HEV antigen using a commercial ELISA (HEV-Ag ELISA, Fortress Diagnostics, Antrim, Northern Ireland, UK) according to the manufacturer's recommendations. We considered any samples with a S/CO ratio >1.0 as initially reactive requiring repeat testing. Samples with a S/CO ratio >1.0 on repeat testing were labelled as repeat reactive (RR). These RR samples were then subject to a neutralisation assay to confirm the specificity of the result using a recently published method (Ankcorn*, et al* 2018a).

Data analysis

We tested whether gender, age, haematological diagnosis, remission status, numbers of treatment lines, numbers of HEV-unscreened blood transfusions received in the previous 5 years, recent treatment with rituximab within 6 months and a transfusion within 28 days had an association with being HEV IgG positive. Differences being HEV IgG positive between these categorical data factors were tested firstly as univariables using Fisher's exact test. The variables age and transfusion history were also considered as a linear variable with the odds ratio presented as an effect for every 10 years or 10 transfusions, respectively. We then used a multivariable logistic regression model to examine factors associated with a positive HEV IgG result. The model was built in a stepwise fashion, adding variables in order of biological importance including a priori factor gender, and considering possible collinearity between factors. The final model included age (linear), gender, underlying haematological disease, numbers of transfusions (linear) and numbers of lines of treatment. All statistical analysis was performed in STATA 13.1 SE.

Results

Patient characteristics

The characteristics of the 1591 patients tested for HEV RNA are detailed in Table 1. Most patients had underlying lymphoma (34.9%) or a plasma cell dyscrasia (32.7%), but the cohort also included 260 patients (16.3%) with chronic leukaemia and 130 patients (8.2%) with acute leukaemia. The majority were within five years of diagnosis of the haematological disorder (67.1%) and either in complete or partial remission (65.2%). Four hundred and eighty patients (30.2%) were lymphopaenic (<1.2 x 10^9/L); seventy five (4.7%) were neutropaenic (<1.0 x 10^9/L). One fifth (330/1591, 20.7%) of the cohort had received a prior autograft; 286 patients with a plasma cell disorder, 40 with underlying lymphoma and 4 had been historically treated with an autograft for acute leukaemia.

Most patients had been treated with at least one course of treatment (78.2%); over half of the patients (55.9%) tested had received a small molecule immunomodulator or monoclonal antibody for their haematological disorder.

Over a third had received immunosuppressive chemotherapy in the preceding six months (39.6%) (Table 2 and table S1, supplementary material); 129 of the total cohort had received rituximab therapy in this time period.

Of the patients tested, haematology and biochemistry blood results were available for 99.9% of patients, 93.2% of which were within 14 days of the HEV RNA test. Overall 205 patients (12.9%) had an abnormal ALT value (>35 IU/L for women, >50 IU/L for men) and 58 (3.6%) had an abnormal bilirubin (>20 µmol/L) at the time of screening for HEV infection.

Transfusion exposure to HEV infection

A total of 441 patients (27.7%) had been transfused with at least one blood component within the hospital trust in the five years prior to enrolment (Figure 1). Of those with a history of transfusions the mean number of transfusions was 15.5 (range 1-346); the majority of transfusions were either packed red cells (53.5%) or platelets (43.4%). As previously stated

all transfusions included in the analysis were transfused before April 2017 and were therefore unscreened for HEV.

HEV RNA prevalence and HEV infected patients

Of the 1591 patients tested for HEV RNA, two viraemic patients were identified giving a prevalence of 0.13% (95% CI, 0.02-0.45%). Both patients harboured a genotype 3c virus.

Both viraemic patients had underlying progressive Multiple Myeloma, diagnosed over 8 years and 16 years previously, and had received immunosuppressive treatment (Bortezomib + Dexamethasone + Panabinostat and Lenalidomide + Cyclophosphamide + Dexamethasone) within five weeks of HEV testing. Neither patient had extensive transfusion histories in the five years previous; patient 1 had received three packed red cells between 23-128 weeks prior to HEV screening and patient 2 had received no transfusions prior to HEV screening. Both patients died during follow-up of their underlying haematological disorder and did not receive any treatment for HEV infection

At the time of screening both patients had unremarkable bloods for ALT, white cell count, neutrophils and platelets; however patient 2 was lymphopaenic (0.39×10^9/L). The two patients had remarkably different virological profiles; the HEV infection in patient 1 was detected during established infection when the plasma viral load was quantified at 7.9×10^4 IU/ml with detectable plasma anti-HEV IgM (S/CO 1.69) and IgG (S/CO 20.32). In contrast patient 2 was in the early phase of HEV infection when the plasma viral load was below the limit of quantitation (<1.0E+2 IU/ml) and there was no detectable plasma anti-HEV IgM (S/CO 0.03) or IgG (S/CO 0.06). It is notable that during follow-up over 13 weeks patient 2 seroconverted for anti-HEV IgM and IgG however the ALT value remained within the normal range and patient 1 only had two of eight ALT readings outside the normal range during follow-up despite high level viraemia. The full diagnostic markers are displayed in figure 2.

Anti-HEV IgG seroprevalence

The overall anti-HEV seroprevalence was 16.7% which rose with age (20.4% in those over the age of 60 years).

The odds of a patient being seroreactive for anti-HEV increased with age and the numbers of red cells/platelet transfusions in the univariable analysis. The odds reduced with increasing numbers of lines of treatment and was also influenced by underlying disease and disease status. Treatment with rituximab in the prior six months did not influence anti-HEV IgG status (OR 1.09, 95% CI 0.68-1.74, p=0.727) (Table 3). A recent transfusion in the preceding 28 days was statistically significant in the univariable analysis but had no effect on the odds of a patient being sero-reactive in the multivariable model. In the final adjusted multivariable analysis increasing age and underlying haematological disease were the strongest factors associated with a patient being seropositive, such that patients with plasma cell disorders were least likely to be seroreactive, whilst patients with acute leukaemia had the highest odds. For every ten years increase in age the odds of a patient being seroreactive increased by 37% (OR 1.37, 95% CI 1.24-1.52) and for every ten transfusion episodes the likelihood of a patient being seroreactive increased by 11% (OR 1.11, 95% CI 1.02-1.20).

HEV Antigen detection

Of the 1591 samples tested, 22 had an initial reactive result (S/CO > 1.0) of which nine were reactive on repeat testing. All nine samples were subjected to neutralisation, only one of which was neutralised therefore confirming a specific result; this was the sample harbouring HEV RNA from one of the two viraemic patients. The HEV-Ag ELISA failed to detect the other viraemic patient at screening (HEV-Ag S/CO 0.04) when the viral load was below the limit of quantitation but subsequent samples from this patient taken 17 days later were reactive in the assay (HEV-Ag S/CO 17.31) when the viral load had risen (3.90E+5 IU/ml).

Therefore the assay was highly specific (99.5%, 95% CI 99.01-99.78), however we were unable to assess sensitivity due to the limited numbers of viraemic patients.

Discussion

HEV is a common viral infection, acquired within the UK as a foodborne zoonosis, resulting in hundreds of thousands of infections each year (Hewitt*, et al* 2014). An increasing number of reports describe persistent HEV infections in the context of haematological malignancy yet

there are no systematic prevalence studies outside the HSCT setting. We aimed to assess the prevalence of HEV viraemia in a cohort of patients with haematological malignancies prior to, or not requiring, HSCT to determine the need for screening.

In this cohort, predominantly of patients with lymphoma and plasma cell dyscrasias, despite using non-HEV screened blood products there was a very low prevalence of HEV viraemia (0.13%, 95% CI 0.015-0.45%), only slightly higher than found in healthy blood donors (0.04%) (Hewitt*, et al* 2014). In comparison, prevalence rates of HEV viraemia as high as 2.4% are reported in allo-HSCT recipients (Ankcorn*, et al* 2018b, Versluis*, et al* 2013). Patients with haematological malignancy outside allogeneic stem cell transplantation are a heterogeneous group of patients with differing levels of immunosuppression which typically vary considerably throughout a therapeutic schedule. In the absence of T-cell subsets and immunoglobulin levels we characterised immunosuppression using surrogates of recent treatment history and absolute neutrophil and lymphocyte counts. A significant number (232 patients) had received moderate to highly intensive chemotherapy in the preceeding six months and nearly a third of patients were lymphopaenic. However, many patients in our cohort were on relatively novel agents and the degree of immunosuppression induced by these agents is not known.

Both HEV viraemic patients identified were under active treatment for multiple myeloma (2/521, 0.38%) and both developed persistent HEV infection; this may suggest an increased risk of developing persistent HEV in multiple myeloma patients compared to other malignancies. We also observed a low anti-HEV IgG seroprevalence in patients with plasma cell dyscrasias and patients with increasing numbers of lines of treatment even after correcting for other factors in multivariable analysis. This could be due to lower prior HEV exposure, but plausibly the loss of anti-HEV IgG related to underlying disease and treatment; either would likely render this group more susceptible to primary HEV infections or even reinfection (Riches and Hobbs 1979).

In the three month follow-up period both viraemic patients had normal ALT values at most time points. This makes it difficult to clinically diagnose HEV infections in such patients. The absence of raised liver enzymes is particularly intriguing; most cases of persistent infection

described have modestly raised liver enzymes, which may be due to ascertainment bias. The significance of HEV viraemia in the absence of raised liver enzymes is unknown, particularly with regard to the risk of chronic liver disease and merits further study.

We tested all patients for anti-HEV IgG to characterise HEV exposure. The most influential factors in the multivariable analysis influencing IgG seroreactivity were increasing age and the underlying haematological diagnosis. We also observed a linear relationship between the numbers of transfusions received and the likelihood of being IgG seroreactive, which remained even after controlling for a recent transfusion, suggesting transfusional HEV acquisition. However, the lack of current active infections indicates that HEV clearance is the norm. Neither the source nor the timing of HEV infection in patients seroreactive for anti-HEV IgG can be demonstrated by our data.

Given the rarity of HEV viraemia (2/1591) we could not assess the sensitivity of HEV-Ag detection, but it has previously been described to be high for persistent infections (Ankcorn*, et al* 2018b, Behrendt*, et al* 2016). The assay failed to detect one viraemic patient in this study who had a very low viral load in the early stage of infection, however the assay was highly specific in this cohort (99.5%).

There are several limitations to this cross-sectional study. This study reflected a typical patient balance in a large tertiary haemato-oncology unit thereby providing a clinically relevant insight into HEV risk in this cohort, however due to the relative rarity of certain conditions such as MDS (n=77) it was not possible to test large numbers of specific disease groups. We were unable to record patients transfusion history at other hospitals outside our centre, therefore we may have underestimated patients' transfusion burden who receive care including transfusions at their local hospital. Finally, dietary acquisition was not subject to systematic assessment and a formal look-back was not undertaken on the two HEV infected patients to ascertain the source of infection.

In conclusion we identified a very low rate of active HEV infection (0.13%) in two patients with advanced myeloma from a heterogeneous cohort of patients under follow-up for haematological malignancy receiving non-HEV-screened blood products. This does not

support the need for routine unselected screening of patients. It also suggests that provision of HEV-screened blood will provide only marginal benefit to this cohort, however we did not characterise the morbidity or mortality associated with acute infection which could be significant. Health economic analyses for the introduction of universal screening for HEV for all patients will need to take into account the low prevalence of persistent HEV in these patients. Notwithstanding the provision of HEV-screened blood for all patients in the UK, the predominant risk of HEV acquisition for the majority of patients is dietary (Tedder*, et al* 2017). Exposure through diet is known to fluctuate widely at a population level due to unidentified factors, therefore individual dietary advice is of paramount importance for all immunocompromised individuals (Adlhoch*, et al* 2016, Ijaz*, et al* 2014). A high level of awareness of HEV amongst clinicians caring for such patients must be maintained due to the risk of severe and fatal courses of HEV infection (O'Gorman*, et al* 2018, Pfefferle*, et al* 2012).

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Figures & Tables

Table 1. Clinical details of patients screened for HEV

Characteristic	No. (%)
Sex	
Male	886 (55.7)
Female	705 (44.3)
Age, yrs, Median [IQR]	65.8 [54.9–73.8]
Underlying Haematological Disorder	
Lymphoma	556 (34.9)
Plasma Cell Disorder[a]	521 (32.7)
Chronic leukaemia	260 (16.3)
Acute leukaemia	128 (8.0)
Myelodysplastic syndrome	77 (4.8)
MPN	36 (2.3)
Aplastic Anaemia	13 (0.8)
Time since diagnosis, yrs	
<1	363 (22.8)
1-5	704 (44.2)
>5	524 (32.9)
Disease status	
Complete or partial remission	1037 (65.2)
No remission	446 (28.0)
Progressive disease	108 (6.8)
Treatment of underlying disease	
No treatment	347 (21.8)
1-2 lines	908 (57.1)
>2 lines	336 (21.1)
Prior Autograft	330 (20.7)
Prior small molecule inhibitors or monoclonal antibodies	889 (55.9)
Immunosuppressive therapy in prior 6m[b]	628 (39.5)
Rituximab in prior 6m	129 (8.1)
Transfusions in 5 yrs prior[c]	
Nil	1150 (72.3)
1-10	292 (18.4)
11-20	46 (2.9)
21-50	70 (4.4)
>50+	33 (2.10)
Blood results, Median [IQR]	
ALT (IU/L)	22 [16 – 31]
Bilirubin (µmol/L)	7 [5 -10]
Total WCC (x 10^9/L)	6.1 [4.4 – 8.3]
Neutrophils (x 10^9/L)	3.4 [2.3 – 4.6]
Lymphocytes (x 10^9/L)	1.6 [1.1 – 2.4]
Platelets (x 10^9/L)	198 [149 – 245]

[a]Includes Multiple Myeloma (n=460) and other plasma cell disorders (n=61).

[b]For treatments given in the preceeding six months see tables 2 and S1.

[c]Transfusions only recorded if within the centre in the five year period prior to enrolment excluding time between 10/4/17 and enrolment (HEV screened products given after that 10/4/17 at the centre).

Abbreviations: IQR, interquartile range; m, months; MPN, Myeloproliferative neoplasm; yrs, years.

Table 2. Details of immunosuppressive medication given to 628 patients in the six months prior to HEV RNA testing.

Treatment	No.
Plasma Cell Disorders, n=271	
High intensity chemotherapy[a]	45
Standard intensity chemotherapy[b]	138
Other combination chemotherapy	1
Single agents +/- corticosteroid	84
CAR-T therapy	1
Radiotherapy	2
Acute Leukaemia, n=75	
High intensity chemotherapy[c]	53
Low intensity chemotherapy[d]	22
Chronic Leukaemia, n=67	
Low intensity chemotherapy[e]	25
Single agents - targeted small molecule inhibitors	41
Single agents - monoclonal antibodies	1
Lymphoma, n=171	
High intensity chemotherapy[f]	34
Moderate intensity chemotherapy[g]	99
Low intensity chemotherapy	26
Single agents - targeted small molecule inhibitors	4
Single agents - monoclonal antibodies	7
Radiotherapy	1
MDS, n=15	
Low intensity chemotherapy	13
Single agents – monoclonal antibodies[h]	2
MPN, n=27	
Low intensity chemotherapy PLUS targeted small molecule inhibitor	3
Low intensity chemotherapy	4
Very low intensity chemotherapy	6
Single agents - targeted small molecule inhibitors	14
Aplastic Anaemia, n=2	
Single agent immunosuppression	2
Total	**628**

Where patients were in clinical trials, if the trial drug administered was known this was recorded, in blinded randomised trials only the known backbone drugs were recorded. The commonest regimes in each category are given below; a full version of this table with all chemotherapy regimes in each category is available in the supplementary material.

[a]e.g. autograft and DTPACE.

[b]e.g. combination chemo PLUS –imid drug/proteasome inhibitor PLUS corticosteroid.

[C]e.g DA-based regimes, FLA(G)-IDA and MidAC.

[d]e.g. low dose ARA-C and azacitidine..

[e]e.g. Rituximab+idelalisib, FLAIR clinical trial.

[f]e.g. R-CHOP+HD MTX, R-CODOX-M+R-IVAC, ABVD+BEACOPP

[g]e.g. ABVD, R-CHOP, R-Bendamustine.

[h]Alemtuzumab in both cases.

Abbreviations: ABVD, Doxorubicin, Bleomycin, Vinblastine, Dacarbazine; ARA-C, Cytarabine; BEACOPP, Bleomycin Etoposide Doxorubicin Cyclophosphamide Vincristine Procarbazine Prednisolone; DA, daunorubicin, cytarabine; DTPACE, dexamethasone, thalidomide, cisplatin, doxorubicin; FLA(G)-IDA, Fludarabine cytarabine, Idarubicin; HD MTX, High Dose Methotrexate; MiDAC, mitoxantrone, cytarabine; R-CODOXM/R-IVAC, Vincristine, Doxorubicin, Cyclophosphamide, Cytarabine, methotrexate, Ifosfamide, Etoposide; R-CHOP, Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone

Table 3. Univariable and multivariable analysis of factors affecting likelihood of a patient being anti-HEV IgG seroreactive

Factor		HEV IgG positive[a] /total (%) n=1591	Univariable analysis OR (95% CI)	Univariable analysis P value[c]	Multivariable analysis[b] OR (95% CI)	Multivariable analysis P value[c]
Sex	F	110/705 (15.6)	-		-	
	M	156/886 (17.6)	1.16 (0.89-1.51)	0.287	1.08 (0.82-1.43)	0.572
Age, yrs	<40	17/165 (10.3)				
	40-59	46/413 (11.1)				
	60-79	149/836 (17.8)		<0.001		
	>80	54/177 (30.5)				
	Effect per 10 years	-	1.37 (1.24-1.51)		1.37 (1.24-1.52)	<0.001
Diagnosis						
	Plasma Cell Disorder	53/521 (10.2)	-		-	
	Acute leukaemia	34/128 (26.6)	3.19 (1.97-5.18)		2.28 (1.25-4.16)	
	Chronic leukaemia	54/260 (20.8)	2.31 (1.53-3.50)	<0.001	1.75 (1.12-2.75)	<0.001
	Lymphoma	86/556 (15.5)	1.62 (1.12-2.33)		1.45 (0.97-2.18)	
	MDS	23/77(29.9)	3.76 (2.14-6.62)		2.41 (1.28-4.54)	
	MPN	15/36 (41.7)	6.31 (3.07-12.97)		4.13 (1.94-8.78)	
	Aplastic Anaemia	1/13 (7.7)	0.74 (0.09-5.77)		0.25 (0.01-4.36)	
Disease status						
	No remission	96/446 (21.5)	-			
	Complete Remission	115/678 (17.0)	0.74 (0.55-1.01)	0.001	-	-
	Partial remission	42/359 (11.7)	0.48 (0.33-0.72)			
	Progressive disease	13/108 (12.0)	0.50 (0.27-0.93)			
Lines of treatment						
	0	70/347 (20.2)	-		-	
	1	117/614 (19.1)	0.93 (0.67-1.30)	<0.001	1.17 (0.80-1.72)	0.017
	2	50/294 (17.0)	0.81 (0.54-1.21)		1.11 (0.71-1.74)	
	>2	29/336 (8.6)	0.37 (0.24-0.60)		0.56 (0.33-0.95)	
Transfusions[d]						
	0	187/1150 (16.3)				
	1-10	41/292 (14.0)				
	11-20	10/46 (21.7)	-	0.002		
	21-50	13/70 (18.6)				
	>50	15/33 (45.5)				
	Effect per 10 transfusions	-	1.11 (1.03-1.19)		1.11 (1.02-1.20)	0.015
RTX within last 6m						
	Y	23/129 (17.8)	1.09 (0.68-1.74)	0.727	-	-
	N	243/1462(16.6)	-			
Transfusion <28d[e]						
	Y	24/86 (27.9)	2.02 (1.24-3.30)	0.005	-	-
	N	242/1505 (16.1)	-			

[a]Any sample with a S/CO >1.1.

[b]Univariable analysis p values were calculated using Fisher's exact test, multivariate analysis p values were calculated using logistic regression.

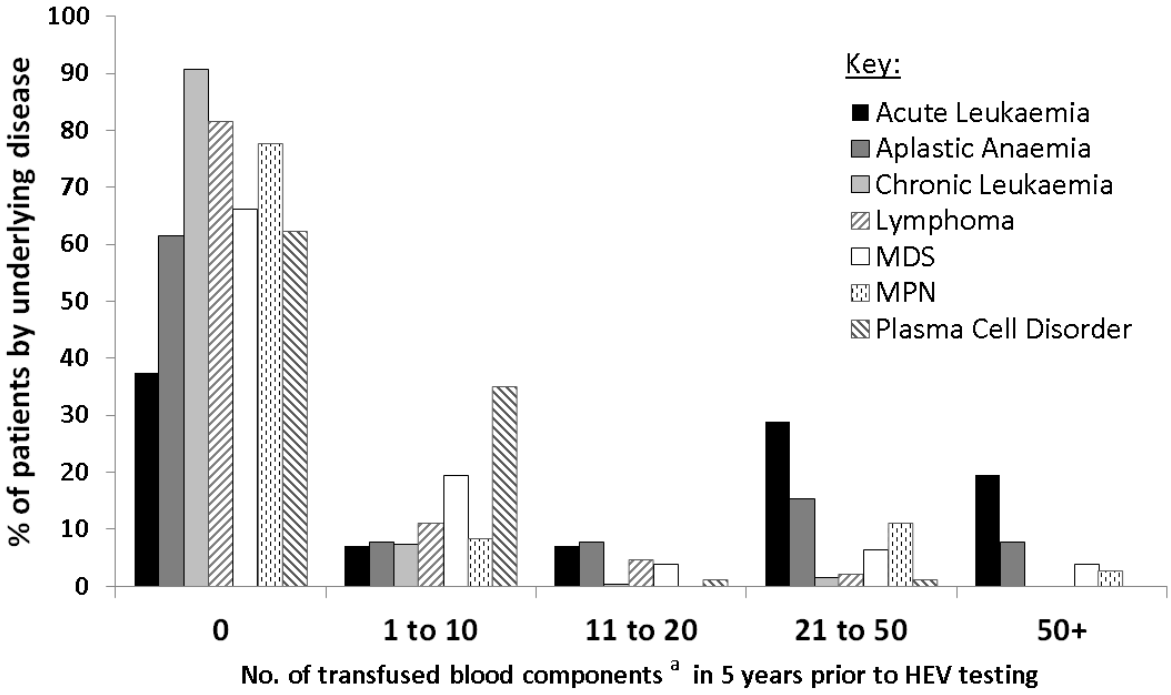
[c]The final multivariable model included age (linear), gender, underlying haematological disease, numbers of transfusions (linear) and numbers of lines of treatment.

[d]Transfusion data only considered when given in 5 years prior and prior to introduction of universal screening.

[e]All transfusions considered including those given after introduction of universal screening.

Abbreviations: CI, confidence interval; D, days; M, months; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; RTX, rituximab.

Figure 1. Proportion of patients receiving HEV unscreened transfusions in the five years prior to HEV RNA testing by diagnosis.



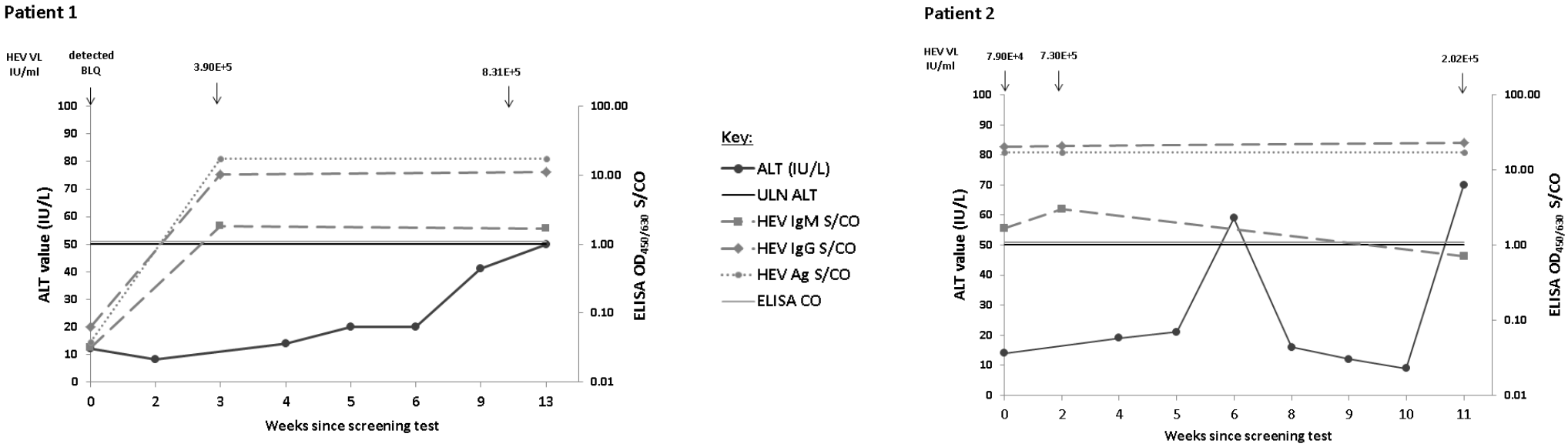
[a]Transfusions only recorded if within five years preceeding HEV testing and before the introduction of the universal screening of blood donations for HEV (10th April 2017). Patients with acute leukaemia with

no transfusions were either transfused after the introduction of universal screening or were in long term remission and transfusions may have occurred prior to the 5 year cut-off.

Abbreviations: MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; no., number.

Figure 2. Diagnostic markers over course of infection in two HEV viraemic patients identified by screening.

	ALT IU/ml (range 10-35)	Bilirubin µmol/L (range 0-20)	WCC x 10^9/L (range 3-10)	Neutrophils x 10^9/L (range 2-7.5)	Lymphocytes x 10^9/L (range 1.2-3.65)	Platelets x 10^9/L (range 150-400)
Patient 1	14	9	6.85	4.84	1.53	145
Patient 2	12	28	3.31	2.40	0.39	102



The secondary y axis represents values for the anti-HEV IgM, anti-HEV IgG and HEV-Ag ELISA assays.

Abbreviations: ALT, alanine aminotransferase; ELISA, enzyme-linked immunosorbent assay; S/CO, sample over cut-off of optical density values; HEV Ag, hepatitis E virus antigen; ULN, upper limit of normal; CO, cut-off; BLQ, below the limit of quantitation; IU, international units; VL, viral load.

Table S1. Details of select immunosuppressive medication for 628 patients treated in the six months prior to HEV RNA testing.

Medication	No.
Corticosteroids	294
Thalidomide/Pomalidomide/Lenalidomide	124
Monoclonal antibodies	
Rituximab	129
Gemtuzumab	15
Alemtuzumab	1
Brentuximab	8
Obinutuzumab	1
Daratumumab	4
Targeted small molecule inhibitors:	
Ruxolitinib/Ibrutinib/Idelalisib/Nilotinib/ Ponatinib/Gilterinib/Imatinib/Quizartinib	77
Bortezomib/Ixazomib/Carfilzomib	146
Other immunosuppressive agents:	
Cyclophosphamide	158
Methotrexate	40
Ciclosporin	2

Here are presented the numbers of patients receiving select specific agents in the six months prior to HEV testing irrespective of whether they were given in combination with other agents. Includes clinical trial patients when the agent administered was known. In cases of blind randomisation, if the drug administered was not known only the backbone drugs were included.

Abbreviations: no., number.

Table S2. Details of immunosuppressive medication given to 628 patients in the six months prior to HEV RNA testing (detailed version of table in main manuscript).

Treatment	No.	Included regimes
Plasma Cell Disorders, n=271		
High intensity chemotherapy	45	Autograft, DTPACE
Standard intensity chemotherapy	138	Ixasomib+lenalidomide+dexamethasone, bortezomib+cyclophosphamide+dexamethasone, bortezsomib+dexamethasone+melphalan, bortezomib+pomalidomide+dexamethasone, bortezomib+thalidomide+dexamethasone, clinical trials (BELLINI, ARROW, Cardamon,CA204004/LDE3+, ITD Myeloma XII, MUK FIVE, MUK SEVEN, M14-031 trial, MMY3010 TRIAL, PADIMAC)
Other combination chemotherapy	1	Carboplatin-based chemotherapy for non-haematological malignancy
Single agents +/- corticosteroid	84	Cyclophosphamide, lenalidomide, bortezomib, carfilzomib, ixazomib, CC-220
CAR-T therapy	1	-
Radiotherapy	2	-
Acute Leukaemia, n=75		
High intensity chemotherapy	53	DA60+GP/ARA-C, DA60+GO, DA50/ARA-C, FLAG-IDA, FLA, MidAC, Mini-FLAG-Ida+Gilterinib, clinical trials (UKALL14. UKALL 2011. UK11. UK60. AML19. AML17)
Low intensity chemotherapy	22	Low dose ARA-C, azacitidine, arsenic, imatinib, nilotinib, clinical trials (WT1 Trial Leukopheresis, IL3RA: KHK2823)
Chronic Leukaemia, n=67		
Low intensity chemotherapy	25	FC-R, ABVD, R-CVP, Rituximab+idelalisib+/-venetoclax, obinutuzumab+chlorambucil, ADCT 402+CHOP-R, venetoclax+idelalisib, ritxuximab+chlorambucil, clinical trials (FLAIR), single agents (cyclophosphamide, cladrabine, chlorambucil, methotrexate)
Single agents - targeted small molecule inhibitors	41	Idelalisib, ibrutinib, imatinib, nilotinib, dasatinib
Single agents - monoclonal antibodies	1	Rituximab
Lymphoma, n=171		

High intensity chemotherapy	34	IVE+/-GDP, ABVD+escalated BEACOPP, MAXI CHOP-R+/-ARA-C, CHOP-R+HD MTX , CHOP-R+bendamustine, ESHAP+brentuximab+bendamustine, R-CODOX-M+/-R-IVAC
Moderate intensity chemotherapy	99	ABVD, R-CHOP, R-CVP, FC-R, DHAP-R, ESHAP, R-BAC, R-bendamustine, R-GDP, R-GemOX, R-CVP+CHOP-R, ESHAP, MATRix, PMit-R, R-HDTMX, R-GDP
Low intensity chemotherapy	26	Chlorambucil, methotrexate, gemcitabine, hydroxycarbamide, ciclosporin
Single agents - targeted small molecule inhibitors	4	Ibrutinib and clinical trials (TAK-659 TRIAL)
Single agents - monoclonal antibodies	7	Rituximab, brentuximab, denusomab for non-haematological malignancy (n=1)
Radiotherapy	1	-
MDS, n=15		
Low intensity chemotherapy	13	Azacitidine, ciclosporin
Single agents - monoclonal antibodies	2	Alemtuzumab
MPN, n=27		
Low intensity chemotherapy PLUS targeted small molecule inhibitors	3	Ruxolitinib PLUS azacitidine or thalidomide
Low intensity chemotherapy	4	Azacitidine
Very low intensity chemotherapy	6	Hydroxycarbamide
Single agents - targeted small molecule inhibitors	14	Ruxolitinib
Aplastic Anaemia, n=2		
Single agent immunosuppression	2	Prednisolone, ciclosporin
Total	**628**	

Where patients were in clinical trials if the trial drug administered this was recorded, in blinded randomised trials only the known backbone drugs were recorded.

Abbreviations: ABVD, Doxorubicin, Bleomycin, Vinblastine, Dacarbazine; ARA-C, Cytarabine; BEACOPP, Bleomycin Etoposide Doxorubicin Cyclophosphamide Vincristine Procarbazine Prednisolone; CAR-T, chimeric antigen receptor T cell therapy; DA, daunorubicin, cytarabine; DHAP-R, Dexamethasone, high dose cytarabine, Cisplatin, Rituximab; DTPACE, dexamethasone, thalidomide, cisplatin, doxorubicin; ESHAP, Etoposide, Methylprednisolone, Cytarabine, Cisplatin; FC-R, Fludarabine, Chlorambucil, Rituximab; FLA(G)-IDA, Fludarabine cytarabine, Idarubicin; HDAC, high dose cytarabine; HD MTX, High Dose Methotrexate; IVE, Ifosfamide, Epirubicin, Etoposide; MATRIx, Methotrexate, Cytarabine, Thiotepa, Rituximab; MiDAC, mitoxantrone, cytarabine; PMitR, Prednisolone, Mitoxantrone, Rituximab; R-CHOP, Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone; R-CVP, Rituximab, Cyclophosphamide, Vincristine, Prednisolone; R-BAC, Rituximab, bendamustine, Cytarabine; R-CODOXM/R-IVAC, Vincristine, Doxorubicin, Cyclophosphamide, Cytarabine, methotrexate, Ifosfamide, Etoposide; R-GemOX, Gemcitabine, Oxaliplatin, Rituximab.