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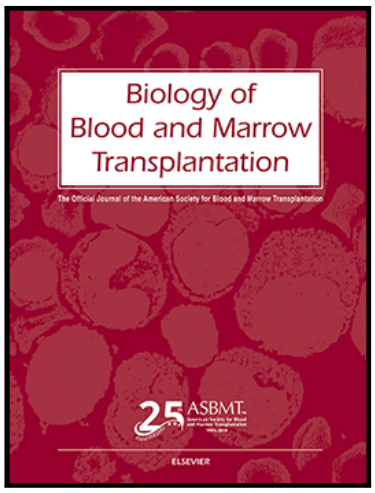


# HEV infection in an Italian cohort of HSCT recipients: seroprevalence and infection

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## Highlights

- Chronic HEV infection in HSCT recipients is an emerging problem of unknown extent
- 6% HEV-IgG positivity was found in our cohort in pre-HSCT screening, and it increased with recipient's age
- 2 patients were diagnosed with chronic HEV hepatitis with HEV-RNA performed after ALT increase and both were HEV-IgG negative
- HEV infection in HSCT recipients in Italy is limited and the benefit of screening pre-HSCT seems absent
- HEV-RNA testing is mandatory in case of any ALT increase

**Article Title**

**HEV infection in an Italian cohort of HSCT recipients: seroprevalence and infection**

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**Running title**

**HEV infection in stem cell transplant recipients**

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**KEYWORDS**

Hepatitis E; Hematopoietic stem cell transplantation; Prevalence; Seroepidemiologic studies; Ribavirin;

## Highlights

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**SUMMARY**

Objectives

Chronic hepatitis E virus (HEV) infection in hematopoietic stem cell transplant (HSCT) recipients is an emerging threat. The aim of this study was to provide data on HEV burden in an Italian cohort of HSCT recipients and analyze risk factors for HEV seropositivity.

Methods

This retrospective study reports data from 596 HSCT recipients from years 2010-2019. It included patients transplanted in years 2010-2015 for whom pre- (n=419) and post-transplant (n=161) serum samples were available and tested retrospectively; and patients in whom prospective HEV testing was performed during the standard care: pre-HSCT IgG screening in 144, pre-HSCT HEV-RNA screening in addition to IgG screening in 60 and HEV-RNA testing in case of clinical suspicion of HEV infection in 59 (26 of them included also in the IgG screening cohorts).

Results

Pre-HSCT HEV-IgG positivity was 6.0% (34/563). Independent risk factor for seropositivity was older age (p=0.039). None of 34 HEV-IgG positive patients had detectable HEV-RNA. One case of transient HEV-RNA positivity pre-HSCT was identified through screening. Two patients were diagnosed with chronic HEV hepatitis, and one was successfully treated with ribavirin.

Conclusions

The burden of HEV infection in HSCT recipients in Italy is limited and pre-HSCT screening seems of n benefit. Timely diagnosis of HEV infection with HEV-RNA is mandatory in case of clinical suspicion.

## INTRODUCTION

Hepatitis E virus (HEV) has been recently recognized as a cause of chronic hepatitis in immunocompromised patients, mainly solid organ transplant and hematopoietic stem cell transplant recipients (HSCT) [1-4]. Among 8 main HEV genotypes known to infect humans, genotypes 1 and 2 are exclusively human and are responsible for both sporadic cases and large outbreaks in developing countries, via fecal-oral route or via waterborne infection [4, 5]. HEV genotypes 3 and 4 are zoonotic and prevalent in developed countries. These HEV genotypes have been detected in a wide range of domestic and wild animals, which are believed to play a major role in the human epidemiology of the virus [4, 6]. Furthermore, HEV infections through blood transfusion and vertical transmission have been reported [5, 7, 8]. HEV genotypes 5-8, together with the Orthohepevirus C1 rat virus, can be responsible for sporadic zoonotic human infections [9].

Chronic infection, which is caused by genotypes 3 or 4, may occur in immunocompromised subjects, it may present with extra hepatic manifestations and may rapidly result in liver fibrosis, cirrhosis or failure [1-3, 10]. In particular, in patients with hematological disorders, it has been associated with increased mortality and liver morbidity [10, 11]. In Europe, only genotype 3 has been found as a cause of autochthonous chronic infection [12-14]. While HEV is recognized as an emerging pathogen in Europe, epidemiological data show a wide range of seroprevalence, varying from <3% to > 50%, depending on patients' population, geographical region and the serological assay used [15-17]. The rate of IgG positivity in blood donors in Italy range from 8.7% to 49% [18-20], and a 10-year seroprevalence study reported a 5.38% prevalence rate in healthy individuals willing to receive HIV testing [21].

Recipients of HSCT are at particularly increased risk of chronic HEV infection due to frequent transfusions of blood products and high level of immunosuppression with

potential lack of T cell response necessary to control HEV infection [1, 3, 10, 22-26]. Limited data is available on HEV in HSCT, but previous studies reported the prevalence of HEV infection in HSCT recipients ranging from < 1% to 4%, and HEV IgG seroprevalence up to 34% [3, 27-31]. Currently, there are no data on the prevalence or course of HEV infection in HSCT recipients in Italy.

The aim of this study was to assess the burden of HEV infection overtime in a cohort of Italian HSCT recipients and to identify the risk factors associated with HEV seropositivity.

## Patients and methods

### Patients and study design

This retrospective study comprises data from two cohorts of HSCT recipients: one tested for HEV retrospectively and one prospectively. Patients receiving HSCT between 2010 and 2019 were identified from the database of our HSCT center.

For the retrospective testing cohort, we identified all consecutive patients who received allogeneic HSCT between 01/12/2010 and 31/12/2015 and had serum samples drawn before HSCT and 6-12 months after HSCT for galactomannan testing and stored at -20°C. In these samples, in 2015 and 2016 we performed HEV IgG testing, and in case of IgG positivity, HEV-RNA was tested.

For the second cohort, we carried out a retrospective analysis of results of tests performed prospectively as a standard of care. These test included: a) pre-HSCT HEV-IgG screening introduced for allogeneic and autologous HSCT since 2017; b) pre-HSCT HEV-RNA screening introduced for allogeneic and autologous HSCT since 2018; c) HEV-RNA testing performed in case of clinical suspicion of HEV infection (alanineaminotransferase [ALT] increase persisting > 2 weeks, with or without other altered liver function tests or clinical signs or symptoms). For year 2016, no retrospective

testing was performed and HEV screening has not been implemented yet. Study design in outlined in Figure 1.

The study was performed in accordance with the Helsinki Declaration. The protocol of the study with retrospective HEV testing was approved by the Regional Ethics Committee and all participants signed the informed consent (approval no. 509REG2014), while the second part of the study from 2017 to 2019 was carried out as retrospective analysis of data coming from our routine patient care and the need for specific informed consent was waived by the Regional Ethics Committee (approval no. 155/2019).

HEV assays

HEV-IgG testing was performed with 2 commercial assays: Wantai HEV-IgG ELISA (Wantai Biopharm, Beijing, China) for samples, stored at -20°C, of patients who received HSCT from 2010 to 2015 and HEV-Ab ULTRA (Dia.Pro – Diagnostic Bioprobes s.r.l. Milan, Italy) for serum samples tested since 2017. All tests were performed as recommended by the manufacturers.

Both test results are interpreted as a ratio of the sample OD450nm and the cut-off value (S/Co); values of S/Co < 0.9 indicate a negative sample and value between 0.9 and 1.1 were considered borderline, positive sample is considered for S/Co values > 1.1.

HEV-RNA was extracted from any sample using MagCore HF16 Automated Nucleic Acid Extractor (RBC Bioscience Corp.). Viral RNA was detected with RealStar® HEV RT-PCT kit 1.0 (Altona Diagnostics GmbH, Hamburg) for samples from 2010 to 2017, and with FTD Hepatitis E RNA assay (Fast Track Diagnostics Ltd., Malta) for samples from 2018. The lower limit of detection for each of the viral RNA detection assay was 60 IU/mL and 188 IU/mL, respectively. Samples were considered positive for HEV-RNA if the cycle threshold was < 45 cycles with an exponential amplification curve.

Samples tested positive for HEV- RNA were analyzed to determine genotype by the amplification and sequencing of the open reading frame 1 (ORF1) genomic region, using primers and conditions previously described [32]. Phylogenetic analysis to determine the degree of genetic variability was carried out by the Neighbour-Joining method using Mega Package, version 7.0.

Risk factors for IgG positivity at HSCT

The following variables were evaluated as possibly associated with HEV IgG positivity at HSCT: age (continuous variable), sex, underlying disease, type of HSCT donor, year of HSCT (continuous variable) and the serological assay used.

Statistical analyses

Descriptive statistics were performed by means of median, mean with range or interquartile range (IQR) and percentages. Categorical variables were compared using chi-square test or Fisher's exact test if appropriate. Continuous variables were compared using Mann-Whitney test. The variables associated with HEV IgG positivity at transplant were assessed in a backwards conditional logistic regression model, in which variables with $p \leq 0.2$ in univariate analysis were entered. A p-value $\leq 0.05$ was considered statistically significant. All the analyses were performed with SPSS Statistics 21.0 (IBM Corp., Armonk, NY, USA).



## RESULTS

Patients

Data from 596 patients were analyzed and their characteristics are outlined in Table 1. Most of the patients were male (334, 56%), and their median age was 49 years (range 17-74). Acute myeloid leukemia and myelodysplastic syndrome were the most common underlying diseases (256, 42.9%), followed by acute lymphoblastic leukemia in 139

(23.3%). Most of the patients received allogeneic HSCT (92.5%), mainly from haploidentical donor (332, 55.7%).

Seroprevalence at HSCT

For 419 patients who received allogeneic HSCT between December 2010 and December 2015, pre-HSCT serum samples were available for HEV IgG testing (median 5 days before HSCT; IQR 7-3 days). This represented 93% of all patients undergoing allogeneic HSCT during that period. From January 2017 to February 2019 a total of 144 patients (101 recipients of allogeneic and 43 of autologous HSCT) were prospectively screening with HEV IgG pre-HSCT (median 22 days before HSCT; IQR 34-15 days).

Overall, 34/563 patients tested positive for HEV IgG pre-HSCT with a seroprevalence of 6.04%. The seroprevalence in patients transplanted in years 2010-2015 with Wantai HEV-IgG ELISA was of 4.77% (20/419), and 9.72% (14/144) in those from years 2016-2018 with HEV-Ab ULTRA. Yearly seroprevalence, together with the assay used, is reported in Table 2.

The variables associated with HEV IgG positivity in a univariate model were: age, year of BMT and type of HEV IgG assay used. The multivariate model confirmed the independent role of age (p=0.038) and a borderline role of the year of BMT (p=0.053) (Table 3).

Seroprevalence after HSCT

Post-HSCT plasma samples were available for 161 unselected patients (38.6%) who received HSCT between 2010 and 2015, at a median time of 236 days after HSCT (IQR 182 days; range 60-471). Overall the post-HSCT seroprevalence of HEV was 7/161 (4.34%), including 5 positive and 2 borderline positive results. Among these patients, 2 were already positive pre-HSCT.

In particular, among the 34 patients who tested positive for HEV IgG pre-HSCT, 25 had a post-HSCT blood sample available, of whom 17 (68%) tested positive, while 8 resulted IgG negative. The median testing time was of 201 days post HSCT.

Screening with HEV-RNA

None of the 20 patients from years 2010-2015 who tested positive for IgG had detectable HEV-RNA either before or after HSCT.

In 2018-2019, 60 patients were screened with HEV-RNA pre-HSCT and only one resulted positive. This was a sample from a 46 years old female, who had 22.000 copies/ml of HEV-RNA in a screening sample, confirmed upon retesting the same sample. Subsequent sample drawn a week later was HEV-RNA negative, as was IgG testing. ALT values were normal at screening but had been elevated for 6 months before transplant, and samples from 2 and 4 weeks before screening were negative for HEV-RNA. Thus, this result was considered as a false positive, although transient viremia, which did not result in a chronic infection, could not be ruled out.

Patients with suspected and confirmed HEV infection

In 52 patients HEV infection was suspected and HEV-RNA was performed. In 38 of them HEV serology was requested as well, and resulted negative in all cases.

Two patients had a positive HEV-RNA result and were diagnosed with a chronic HEV infection, with HEV-RNA positivity lasting respectively 8 and 7 months, as determined by retrospective testing of available serum samples. They were IgG negative at the diagnosis of HEV infection. Both had a genotype 3f, with no evidence of significant homology between the strains.

Patient 1 was a 59-year-old male who developed a persistent increase of ALT, thrombocytopenia and leukopenia 2.5 months after autologous HSCT for NHL. He resulted positive for HEV-RNA with 4.518.000 copies/mL in blood and HEV-RNA of

3.978.000 copies/mL in stool. He was not receiving any immunosuppressive therapy. Transmission trough blood was ruled out by testing the residual available blood derivate which resulted negative (RealStar® HEV RT-PCT kit 1.0, Altona Diagnostics GmbH, Hamburg). The patient reported consumption of wild boar meat 8 months before and had ALT level of 70 UI/L. During the follow up, HEV-RNA positivity was confirmed and ALT increased to 520 U/L. Therefore, he was treated with oral ribavirin for 3 months and he achieved sustained virological response (SVR) at 12 and 36 months of follow-up.

Patient 2 was a 56 years old female who underwent haploidentical transplant for acute myeloid leukemia. She was negative for HEV IgG pre-HSCT, and developed persistent ALT increase 3 months after transplant (maximum ALT value 671 UI/L), attributed initially to GvHD and voriconazole treatment. Diagnosis of chronic HEV infection was made 10 months after HSCT, with blood HEV-RNA 814.162 copies/mL, and positive HEV-RNA in a sample drawn 3 months before. The patient died 7 days after HEV diagnosis due to bacterial sepsis and invasive aspergillosis, with signs of ongoing liver failure.



## DISCUSSION

This is the first study to determine the burden of HEV infection in HSCT recipients in Italy. The main results are: a HEV IgG seroprevalence pre-HSCT of 6%, increasing with the age of patients and possibly also with more recent year of transplant, suggesting that HEV might be an emerging infection in this setting, particularly with increasing age of patients to whom HSCT is currently offered. Additionally, two cases of chronic HEV hepatitis were diagnosed, one successfully treated and the other, in whom the diagnosis had been delayed, with the patient dying due to multiple causes, but with concomitant liver failure.

HEV seroprevalence (6%) is in line with a previous report in healthy individuals in Italy [21], but lower than that observed in case of universal screening prior to HSCT in France and the Netherlands, where reported seroprevalence rates ranged from 12% to 34% [3, 27]. Given that the assay used and the patient population were similar to our cohort, differences in seroprevalence rates are possibly due to local prevalence in geographical region. Of note, HEV seroprevalence in our cohort seems to increase from 2011 to 2018, suggesting a possible progressive diffusion of HEV exposure in our region which may warrant even more attention to the problem of HEV in the immunocompromised. Additionally, with a prevalence of HEV infection of 3.4% among patients with ALT increase, our results are in line with other studies on HEV in HSCT which reported the rates of HEV infection ranging from <1% to 4% in such a setting. The available data on HEV in HSCT recipients, reported together with the assay used and the design of the study, are outlined in Table 3 [3, 11, 27-31, 33-35]. In addition to a recent systematic review which reported IgG seroprevalence and rate of infection in HSCT (respectively, 11.4% and 1.5%), we report also data from one pediatric cohort study and two retrospective studies reporting data on 46 HEV infected HSCT recipients [36].

In this study, older age was the only independent risk factor associated with higher seropositivity rate. This association has been already reported in immunocompetent and immunocompromised subjects [25, 37]. It probably stems from a longer exposure to risk factors such as consumption of pork and game meat, especially raw, consumption of raw vegetables, occupational contact with pigs, contact with waste water, all of which were described in case-control studies in cases of acute HEV hepatitis in England and Wales [38], Germany [39] and the Netherlands [40].

Given the peculiar bimodal design of this study, the possible impact of the change in serological assay on the prevalence rate should be also carefully considered. An important differences in sensitivity between serological tests have been reported [41].

Initially, lower sensitivity of the Dia.Pro assay (used between 2016 and 2019 in our study) compared to Wantai has been reported [42]. However, a recent study reported comparable sensitivity between currently marketed kits for HEV IgG detection such as Wantai, Euroimmun, MP, Dia.Pro, DSI, and Mikrogen, and the discrepancy with earlier results may be related to the improvement made in recent versions of the tests [43]. Therefore, there is no clear evidence that the second assay used is significantly more sensitive than the first one, and, in addition, the increase the in seroprevalence was also observed within the period of testing with the same assay (Table 2).

One of the particular risk factors in patients with hematological malignancies, including HSCT recipients, is the chance of HEV transmission through blood products, described both in HEV endemic and non-endemic countries [7, 44-49]. Since 2012, eight EU countries implemented HEV screening strategies [49]. In England a HEV viremia prevalence of 1 in 3,830 donations was reported [7], with similar results in many other European countries, indicating the wide spread of this infection in European human populations [45]. In Italy, HEV screening is not performed in blood donors. In one of our cases, transmission trough blood transfusion was ruled out by testing the residual available blood derivate.

While the screening for chronic hepatitis B and C is a standard of care in HSCT setting, the need for HEV screening remains debatable. In solid organ transplant recipients, HEV screening is considered not needed [50]. Currently, ECIL guidelines recommend performing HEV-RNA in both donors and recipients of HSCT [23]. However, data confirming the benefit and cost-effectiveness of this recommendation are lacking and studies performing HEV- RNA testing at HSCT reported a very low incidence (1/153), even in countries where seroprevalence of HEV infection is high, such as UK [15, 29]. Our study demonstrated that serological screening is unhelpful, since HEV IgG positivity had no impact on the subsequent HEV infection, it waned in some patients and no case of reactivation was observed. Indeed, the possibility of HEV reactivation is elusive as only

three possible cases were published [51-53], but a recent study in an animal model documented the persistence of low titers of HEV-RNA in liver, gallbladder and bile [54]. Although HEV-RNA is superior to serological testing in immunocompromised patients, also RNA screening had no clinical impact, since it yielded only one positive result which was not confirmed subsequently and had no clinical impact. Thus, we share the conclusion of a recently published study in patients with hematological malignancies in the UK, which did not support the need for routine unselected screening for HEV infection in this setting [55].

On the other hand, HEV-RNA testing is the preferred method for diagnosis of chronic infection, and two reported cases of chronic HEV infection had undetectable serology at the time of diagnosis. Thus, we confirm that it is mandatory to test for HEV-RNA all patients with an increase in ALT levels, even when other probable causes of increase in liver enzymes are present. Hepatic GvHD is difficult to distinguish from other liver diseases and it has been reported in a Dutch cohort that 16% of patients with (probably or proven) hepatic GvHD had concomitant HEV infection [31]. Our patient who experienced chronic HEV hepatitis and liver failure, had received a diagnosis of liver GvHD and voriconazole toxicity, while HEV might have contributed to ALT increase. The timely diagnosis of HEV infection in the presence of multiple comorbidities is crucial since a therapeutic option of ribavirin has been shown effective in this setting (Table 4), with a viral clearance rate of about 80% [12, 34, 56, 57]. Additionally, successful treatment of acute HEV infection in patients with hematological malignancies with the aim of preventing a chronic infection has been reported [58].

Limitations of this study include a partially retrospective design and no direct comparison of two serological methods used. However, such a design allowed us to include numerous patients over the years, with high sample representativity (93%), while possible impact of the change in the serological assay might have contributed to the

change of seroprevalence observed over the years. Finally, our result might not be applicable to settings with a very high prevalence of HEV exposure and no testing in blood donors, in whom HEV-RNA screening might be of use.

In conclusion, the burden of HEV infection in HSCT receipts in Italy is limited, and screening with either serological or direct assays seems of no benefit. However, timely diagnosis with HEV-RNA should be mandatory in case of clinical suspicion, mainly based on ALT increase and thrombocytopenia. Studies from other geographical regions and longitudinal observations are warranted to better define the burden of HEV in hematology worldwide.

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**Tables**

**Table 1. Patientsôcharacteristics.**

| **Characteristics** | **All patients*** (n=596) | **Patients who underwent HEV IgG screening at HSCT** (n=563) |
|---|---|---|
| Median age at transplantation, years (range) | 49 (17-74) | 48 (17-74) |
| Sex, male | 334 (56%) | 318 (56.5%) |
| Underlying disease | | |
| Acute myeloproliferative disorders (AML/MDS) | 256 (42.9%) | 237 (42.1%) |
| Acute lymphoproliferative disorders (ALL and aggressive lymphomas) | 139 (23.3%) | 133 (23.6%) |
| Chronic lymphoproliferative disorders (CLL, MM, indolent lymphomas) | 105 (17.6%) | 99 (17.6%) |
| Chronic myeloproliferative disorders (CML and MF) | 66 (11.1%) | 65 (11.5%) |
| Severe aplastic anemia | 28 (4.7%) | 27 (4.8%) |
| Multiple sclerosis | 2 (0.3%) | 2 (0.4%) |
| Type of donor | | |
| Autologous | 45 (7.5%) | 43 (7.6%) |
| Allogeneic | | |
| Matched related | 150 (25.2%) | 142 (25.2%) |
| Matched unrelated | 63 (10.6%) | 60 (10.7%) |
| Haploidentical | 332 (55.7%) | 312 (55.4%) |
| Cord blood | 6 (1%) | 6 (1.1%) |

AML, acute myeloid leukemia, MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; CLL, chronic lymphoid leukemia; MM, multiple myeloma; CML, chronic lymphoid leukemia; SAA, severe aplastic anemia; MS, multiple sclerosis; MUD, mismatched unrelated donor; UCB, umbilical cord blood.

*Including those who underwent HEV IgG screening and additional 33 tested with HEV-RNA because of clinical suspicion of HEV infection.

**Table 2. Distribution of HEV-IgG at HSCT positivity according to the year of transplant**

| Date of BMT | Patients with positive result among those tested with HEV-IgG at HSCT | Seroprevalence, % | Assay |
|---|---|---|---|
| 2011* | 3/83 | 3.6% | Wantai |
| 2012 | 3/86 | 3.5% | Wantai |
| 2013 | 6/90 | 6.7% | Wantai |
| 2014 | 4/90 | 4.4% | Wantai |
| 2015 | 4/70 | 5.7% | Wantai |
| 2017 | 3/61 | 4.9% | Dia.Pro |
| 2018** | 11/83 | 13.3% | Dia.Pro |
| Total | 34/563 | 6.0% | |

*including 3 patients transplanted in the end of December 2010; ** including 6 patients transplanted in the beginning of January 2019.

**Table 3. Univariate and multivariate analysis of risk factors for positive HEV IgG serology in 563 patients from years 2010-2019.**

| | Univariate analysis | | | Multivariate analysis | |
|---|---|---|---|---|---|
| | Positive, n=34 (6%) | Negative, n=529 (94%) | P-value | OR (95% CI) | P-value |
| **Age, median (range), years** | 52 (25-70) | 49 (17-74) | **0.015** | 1.029 (1.002-1.057) | **0.038** |
| **Sex** | | | 0.548 | | |
| Male | 19 (6) | 299 (94) | | | |
| Female | 15 (6.1) | 230 (93.9) | | | |
| **Year of BMT** * | | | **0.024** | 1.196 (0.998-1.434) | 0.053 |
| **Underlying disease** | | | 0.238 | | |
| Acute myeloproliferative disorders | 16 (6.8) | 221 (93.2) | | Ref | 0.395 |
| Acute lymphoproliferative disorders | 4 (3) | 129 (97) | | 0.493 (0.157-1.552) | |
| Others | 14 (6.2) | 213 (93.8) | | 1.082 (0.508-2.306) | |
| **Type of donor** | | | 0.318 | | |
| Autologous | 4 (9.3) | 39 (86.7) | | | |
| Allogeneic | 30 (5.8) | 490 (0.92) | | | |
| **HEV IgG assay** | | | **0.031** | | 0.731 |
| Wantai | 20 (4.8) | 399 (95.2) | | Ref | |
| HEV-Ab ULTRA | 14 (9.7) | 130 (90.3) | | 1.261 (0.337-4.723) | |

OR, odds ratio; CI, confidence interval; *Considered as a continuous variable; see the rate of positive HEV-IgG according to the year of transplant in Table 2.

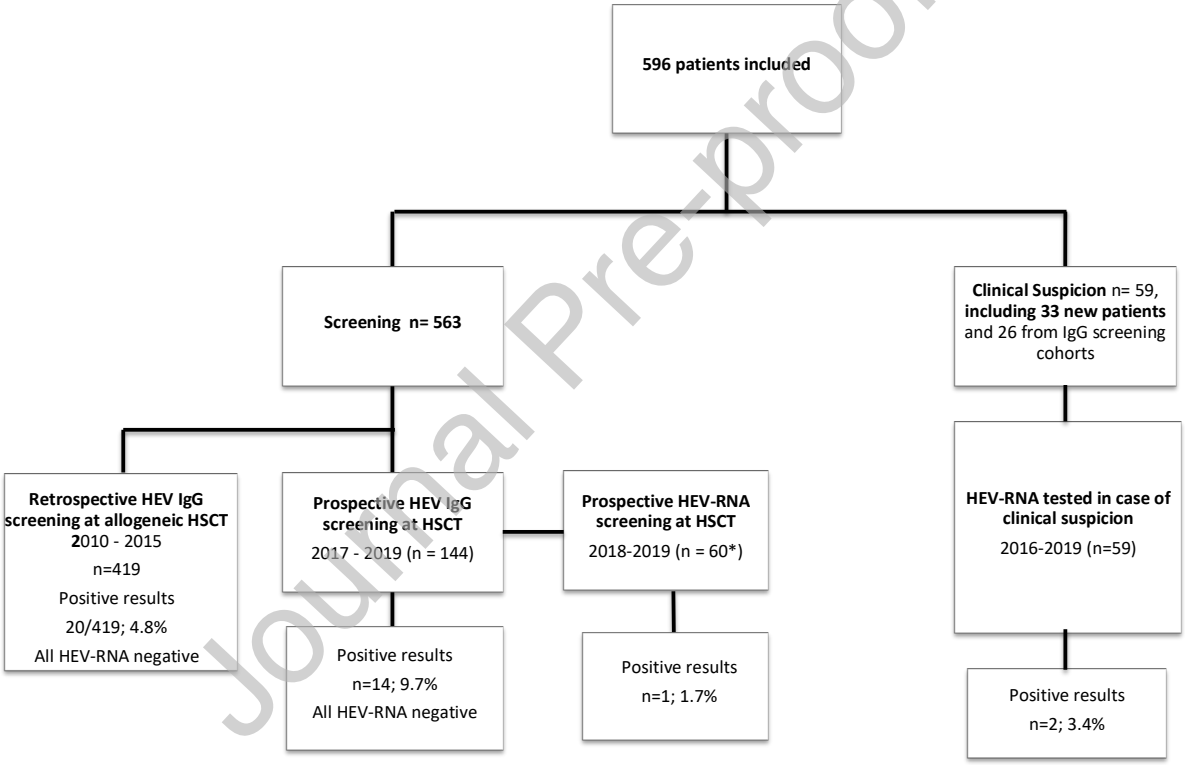
**Table 4. HEV infection prevalence, rates and outcomes in HSCT recipients, divided by study design.**

| **Study design** | **Universal screening** | | | | **HEV testing in case of ALT increase** | | | | **Retrospective cohort study of HEV-infected patients** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Study** | **Reekie 2018[29]** | **Ankcorn 2018[35]** | **Versluis 2013[3]À** | **Abravanel 2012[27]** | **Tang 2019[30]** | **Willemse 2017[31]** | **Jaber 2014[33]** | **Koenecke 2012[28]** | **Xhaard 2019[34]** | **Von Felden 2019[11]** |
| **Country** | UK | England | Netherlands | France | China | Netherlands | Canada | Germany | France | Europe |
| **Type of patients** | AlloHSCT and autoHSCT | AlloHSCT candidates | AlloHSCT candidates | AlloHSCT and autoHSCT candidates | Haploidentical alloHSCT | AlloHSCT | Pediatrics alloHSCT | AlloHSCT | AlloHSCT | Hematological malignancies, including 21 HSCT |
| **Number of patients** | 259 | 144 | 328 | 88 (77 alloHSCT and 16 autoHSCT) | 177 | 130 | 45 | 52 | 25 | 50 |
| **Serology assay used** | ND | ND | Wantai | Adaltis/Wantai | MP Diagnostics | ND | Feldan Bio Inc | Abbot | ND | ND |
| **Years of testing** | 2013-2015 | 2016 | 2006-2011 | 2009-2010 | 2014-2017 | 2005-2015 | 2008-2010 | 1998-2004 | 2010-2015 | 2014-2017 |
| **IgG seroprevalence** | ND | ND | 13% | 12.5%/36.4% | ND | ND | 13.3% | 6% | ND | ND |
| **HEV-RNA positive patients (%)** | 1 (0.39%) | 3 (2.08%) | 8**Â** (2.4%) | 0 (0%) | 7 (3.9%) | 5 (4%) | 0 | 0 (0%) | 25 (NA) | 50 (NA) |
| **SVR achieved/No. of patients treated with ribavirin** | 0/0 | ND | 1/1 | 0/0 | 0/0 | 2*/4 | 0 | 0/0 | 11/13** | 19/24 |
| **Death with HEV-viremia/HEV infected** | 1/1 | ND | 4/8 | 0/0 | 2/7 | 1/5 | 0/0 | 0/0 | 1/25 | 7/50 |

AlloHSCT, allogeneic hematopoietic stem cell transplant recipients; ALT, Alanine aminotransferase; autoHSCT, autologous hematopoietic stem cell transplant recipients; NA, not applicable; ND, no data; SVR, sustained viral response. †: included also 207 samples from the subset of 138 patients tested because of ALT increase, which identified 1 HEV infected patient. * In the third patient HEV-RNA became negative but he died shortly after due to other causes so SVR could not be evaluated; ** SVR could not be evaluated in one of the patients who died due to other causes 2 months after starting ribavirin with decreasing HEV-RNA values.

**Figures**

Figure 1 Flowchart of the study.



HSCT, hematopoietic stem cell transplantation; HEV, hepatitis E virus.

* All included in prospective HEV IgG screening cohort; ** 26 patients included in one of two IgG screening cohorts and all IgG negative, 33 patients not included in screening cohorts.