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Prevalence of anti-hepatitis E virus immunoglobulin G in HIV-infected individuals over three decades

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ABSTRACT

Background: Hepatitis E virus (HEV) genotype 3 is endemic in Europe, and the infection is mostly subclinical or acute and self-limiting. However, persistent infection is described among HIV-infected individuals. The prevalence of antibodies against HEV (anti-HEV) among HIV-infected persons varies geographically and is unknown in Denmark. Rates of co-infection with HEV among HIV-infected individuals in Denmark over three decades, from the early 1980s to 2013, were investigated.
Methods: A total of 2506 HIV-infected persons were investigated from two cohorts followed at Hvidovre Hospital, Denmark. Blood samples were tested retrospectively for anti-HEV, including samples from 2216 persons who were enrolled in a prospective clinical cohort and followed between 1995 and 2013, as well as samples from 290 persons from a historical cohort followed between 1980 and 1994. For anti-HEV seroconverting individuals, serial samples were tested for HEV RNA. Factors associated with anti-HEV status were explored using multivariable logistic regression analysis.
Results: The overall HEV seroprevalence rates were stable during the 1980s, 1990s, and 2000–2013 (23.1%, 22.9%, and 23.7%, respectively). In all decades, rates of anti-HEV increased with older age, and anti-HEV seropositivity was associated with older generations, HIV risk group, and geographic origin. Persistent HEV infection was not detected in any of 57 individuals with anti-HEV seroconversion.
Conclusions: HEV seroprevalence rates were stable in HIV-infected individuals from the early 1980s to 2013. Rates increased with age. No evidence of persistent HEV infection was detected. Infection with HEV is frequent, but persistent HEV infection is rare among HIV-infected individuals.

Introduction

Hepatitis E virus (HEV) genotype 3 (gt3) is endemic in Europe and other industrialized countries, and an increased focus has been placed on infection with this agent over the last decades (Kamar et al., 2014). HEV-gt3 has a zoonotic and mainly porcine reservoir, and the predominant route of transmission is believed to be foodborne (Kamar et al., 2012; Van der Poel, 2014). Parenteral transmission via blood transfusion and organ transplantation has also been reported (Schlosser et al., 2012; Hewitt et al., 2014). HEV-gt3 infection is mostly subclinical or benign and self-limiting. However, persistent infection with HEV-gt3 that might lead to chronic HEV infections has been reported in patients after organ transplantation, in patients with haematological disorders treated with chemotherapy, and among HIV-infected individuals (Dalton et al., 2009; Colson et al., 2011; Kamar et al., 2013). Studies on the prevalence of HEV antibodies (anti-HEV) in HIV-infected individuals vary among geographical areas (Debes et al., 2016). The reported prevalence of anti-HEV in HIV-infected individuals was 2.6% in Switzerland, 9% in the UK, 21% in Spain, and 37.8% in France (Abravanel et al., 2017; Kenfak-Foguena et al., 2011; Keane et al., 2012; Mateos-Lindemann et al., 2014). The prevalence of HEV immunoglobulin G (IgG) among healthy Danish blood donors declined from 32.9% in 2003 to 10–19.3% in 2013 (Christensen et al., 2008; Holm et al., 2015).

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The prevalence of anti-HEV among HIV-infected individuals in Denmark is unknown. The primary aim of this study was to estimate the prevalence of anti-HEV IgG among HIV-infected patients over three decades: the early 1980s, 1990s, and 2000–2013. Secondary aims included an assessment of factors associated with the presence of anti-HEV IgG and the presence of persistent HEV infection in HIV-infected patients.

Materials and methods

Subjects and samples

Patients eligible for the current study were any individual attending the Department of Infectious Diseases, Hvidovre Hospital and under active follow-up from January 1, 1995 to May 30, 2013. All patients were followed prospectively as part of an ongoing nationwide Danish HIV cohort study (Obel et al., 2009). New referrals were continuously enrolled into the cohort. Blood samples were drawn at clinic visits and plasma or serum was stored at −80 °C.

Additional samples from individuals who had attended the clinic in the early 1980s but who had died before 1995 were retrieved from a repository and served as a historical cohort of HIV-infected patients before the era of antiretroviral treatment.

The study was approved by the local ethics committee for the Capital Region of Denmark (No. H-18045809) and the Danish Data Protection Agency (No. 2007-41-1634).

Testing strategy

For individuals in both cohorts, the most recent available serum or plasma sample was tested for anti-HEV IgG. Individuals with anti-HEV IgG-positive results in their latest sample were subsequently tested for anti-HEV IgG in their first available archived sample. For patients with an anti-HEV seroconversion pattern between their first and most recent sample, additional intermediate samples were identified to detect the time of seroconversion. Further, serial samples close to and within 3–12 months before anti-HEV seroconversion were tested for HEV RNA.

Anti-HEV ELISA

HEV antibodies were detected using the Wantai HEV IgG ELISA assay (Nordic BioSite, Copenhagen, Denmark), in accordance with the manufacturer's instructions; a signal/cut-off (S/CO) ratio of ≥1.1 was used to indicate a positive result.

HEV RNA detection

Detection of HEV RNA was done using a CE-marked qualitative nucleic acid amplification test (NAT) (Procleix HEV Assay; Grifols Diagnostic Division, Emeryville, CA, USA) with a 95% detection probability of 7.9 IU/ml and a specificity of 99.95%.

Confirmation of HEV RNA reactivity and genotyping

Initial HEV RNA NAT-positive reactions were confirmed by diagnostic real-time PCR amplifying a 79-bp fragment of ORF-3 (Jothikumar et al., 2006). RNA was extracted using the MagNa Pure LC Total Nucleic Acid Isolation Kit on a MagNa Pure 96 instrument (Roche Diagnostics, Rotkreuz, Switzerland) prior to PCR. Further PCR amplification was attempted for the purposes of genotyping using previously described methods (Midgley et al., 2014; Hogema et al., 2016).

Statistical analysis

Values are presented as the median with interquartile range (IQR) or range. Differences were tested using the Chi-square test or Mann–Whitney test, as appropriate.

Follow-up time was calculated from the time of the first available sample to the latest sample analysed. The incidence rate was calculated from anti-HEV seroconverting patients. For patients who seroconverted, the time to seroconversion was considered half way between the closest negative and positive anti-HEV IgG test.

Factors associated with anti-HEV IgG positivity at baseline or seroconversion during follow-up were studied by multivariable logistic regression analysis and presented as an odds ratio (OR) with 95% confidence interval (CI) among the Hvidovre Hospital clinic cohort.

Persistent HEV infection was defined as HEV viremia in two or more samples taken at least 3 months apart.

HEV seroprevalence rates were calculated among newly enrolled patients in the cohorts by using time-points of the first available positive anti-HEV IgG result and the first available negative anti-HEV IgG result, with the assumption that anti-HEV IgG-negative patients had never been anti-HEV IgG-positive. This assumption may be justified if the mean time between the latest and first samples of anti-HEV-negative patients is limited; otherwise there is a risk of underestimating the true seroprevalence rates, as the level of anti-HEV declines over time. HEV seroprevalence rates were calculated for three time periods: the 1980s, the 1990s, and 2000–2013.

The statistical analyses were performed using IBM SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA).

Results

Figure 1 shows flow charts of the two study cohorts, which included a total of 2506 individuals: 2216 patients under active follow-up and 290 individuals from the historical cohort. In total, 584 (23.3%) individuals were anti-HEV IgG-positive in their most recent sample. Of these, an earlier sample was retrieved for 413 individuals, and 336 samples were also anti-HEV IgG-positive at this time-point. Among the 77 individuals with discordant tests, a total of 276 serial samples between the two time-points were available for 57 individuals. The median interval between the first available sample and the tested (latest) sample was 7 years (IQR 3–13 years) for the anti-HEV IgG-negative patients. Among the patients with anti-HEV IgG-positive results, the median S/CO declined from 7.94 (IQR 3.48–12.23) in the first available samples to 6.74 (IQR 2.85–11.67) in the second samples, over a median interval of 8 years (IQR 4–13 years).

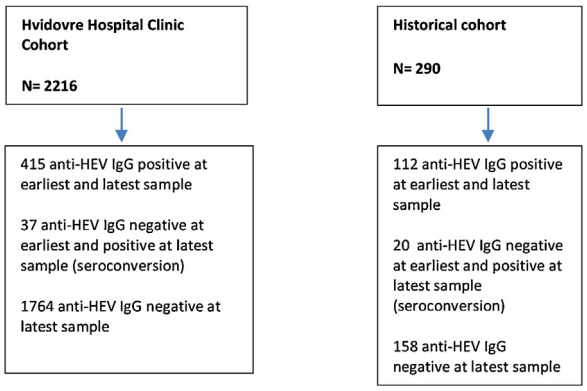

Figure 1. Flow charts for the study cohort.

Table 1
Characteristics of HIV-infected individuals included in the prospective Hvidovre Hospital clinic cohort.

	Anti-HEV IgG-positive *n* = 415 18.7% (CI 17.2–20.4%)	Anti-HEV IgG-negative *n* = 1801 81.3% (CI 79.6–82.8%)	*p*-Value
Age (years), median (IQR)	40 (33–48)	34 (28–41)	0.0001
Male sex, *n* (%)	313 (75.4%)	1339 (74.4%)	0.66
CD4 T-cell count (cells/μl), median (IQR)	280 (122–470) (*n* = 343)	296 (146–478) (*n* = 1467)	0.48
Antiretroviral therapy	34 (9.8%)	176 (8.2%)	0.32
Route of HIV transmission, *n* (%)			
HSX	146 (35.2%)	610 (34.2%)	
IDU	76 (18.3%)	250 (14.0%)	
MSM	164 (39.5%)	842 (47.1%)	
Other or unknown[a]	29 (7.0%)	84 (4.7%)	0.007
Country of origin, *n* (%)			
Europe	338 (81.6%)	1389 (77.4%)	
America	2 (0.5%)	38 (2.1%)	
Africa	42 (10.1%)	246 (13.7%)	
Asia	27 (6.5%)	85 (4.7%)	
Greenland	3 (0.7%)	26 (1.4%)	
Other/unknown	2 (0.5%)	11 (0.6%)	0.03

HEV, hepatitis E virus; IgG, immunoglobulin G; CI, confidence interval; IQR, interquartile range; HSX, heterosexual; IDU, injection drug use; MSM, males who have sex with males.
[a] Other routes of HIV transmission include perinatal infection and transfusion transmission.

Hvidovre Hospital clinic cohort

Patient characteristics are shown in Table 1. Briefly, among 2216 individuals, 415 (18.7%) were anti-HEV IgG-positive at their earliest available test. Anti-HEV IgG-positive individuals were older and more likely to be from Europe or Asia. There were more individuals who had acquired HIV through injection drug use (IDU) and fewer males who have sex with males (MSM) who were anti-HEV IgG-positive. Neither CD4 T-cell count within 90 days of sampling nor the use of antiretrovirals at the time of sampling was associated with anti-HEV IgG status. By multivariable logistic regression analysis, individuals born after 1970 compared to before 1970 and MSM compared to individuals with heterosexual (HSX) HIV transmission were less likely to be anti-HEV IgG-positive. Additionally, individuals from Asia compared to Europe were more likely to be anti-HEV IgG-positive (Table 2).

The follow-up time was not available for 19 of the 1801 individuals without anti-HEV IgG at baseline. Of the remaining 1782 individuals, 37 seroconverted during the 15 836 years of follow-up, corresponding to an incidence rate of 2.34 cases (95% CI 1.67–3.19) per 1000 person-years. The median time to seroconversion from enrolment was 307 days (range 28–946 days). Age, sex, route of HIV transmission, and country of origin were not associated with seroconversion (data not shown).

Historical cohort

Of the 290 individuals, 20 (6.9%) were female and 132 (45.5%) were anti-HEV IgG-positive. Samples were collected between 1980 and 1994. The median age at the time of testing was 34 years (IQR 28–41 years).

HEV RNA

A total of 276 serial samples from 57 anti-HEV seroconverting individuals (37 from the clinic cohort and 20 from the historical cohort) were analysed for HEV RNA. Only one sample from an individual included in the Hvidovre Hospital clinic cohort was HEV RNA NAT reactive, which was confirmed by a second PCR (index sample). Samples drawn 3, 12, and 13 weeks before and 9 weeks after the index sample all tested HEV RNA-negative, showing no evidence of a persistent HEV infection. Anti-HEV IgG was first detected 3 weeks before the index sample, and the S/CO ratio increased with the subsequent samples tested. Anti-HEV IgM was only weakly positive (S/CO 2.3) in one sample taken 9 weeks after the index sample.

Table 2
Factors associated with anti-HEV immunoglobulin G positivity in the prospective Hvidovre Hospital clinic cohort at cohort entry.

Variable	Multivariable OR (95% CI)	*p*-Value
Birth cohort		
1970–	1.0	
1950–69	5.05 (3.44–7.41)	0.0001
>1950	1.96 (1.43–2.68)	0.0001
Route of HIV transmission category		
HSX	1.0	
IDU	1.29 (0.91–1.82)	0.15
MSM	0.72 (0.54–0.94)	0.02
Other or unknown[a]	1.27 (0.79–2.05)	0.33
Country of origin		
Europe	1.0	
America	0.32 (0.08–1.33)	0.12
Africa	0.84 (0.57–1.25)	0.39
Asia	1.65 (1.02–2.67)	0.04
Greenland	0.55 (0.16–1.84)	0.33
Other/unknown	0.87 (0.18–4.10)	0.86

HEV, hepatitis E virus; IgG, immunoglobulin G; HSX, heterosexual; IDU, injection drug use; MSM, males who have sex with males; CI, confidence interval.
[a] Other routes of HIV transmission include perinatal infection and transfusion transmission.

This individual was a 38-year-old male of Danish origin on a three-drug antiretroviral regimen with a CD4 T-cell count >200 cells/μl (nadir CD4 T-cell count 205 cells/μl at the time of the index sample to 299 cells/μl 9 weeks later), who had acquired HIV infection through sex with men. Liver enzymes were slightly elevated both prior to and after anti-HEV seroconversion, and the patient notes did not indicate a symptomatic HEV infection. The level of HEV RNA was below the limit for further characterization by sequencing.

Anti-HEV IgG prevalence by decade, age group, and year of birth

Anti-HEV IgG seroprevalence rates for newly enrolled patients in the cohorts and calculated from the results of the first available sample categorized in time periods of the 1980s, the 1990s, and 2000–2013 were 23.1%, 22.9%, and 23.7%, respectively ($p = 0.89$) (Figure 2). By stratifying patients into 10-year age groups, a gradual

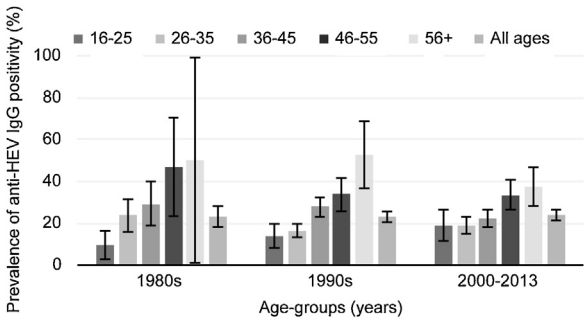

Figure 2. Prevalence of anti-hepatitis E virus immunoglobulin G by decade and age-group.

increase in HEV seroprevalence rate was observed from the youngest to the oldest patients in all three decades ($p < 0.003$ for each decade). There was a tendency, although statistically non-significant, towards a decreasing anti-HEV prevalence rate from the 1980s to the latest decade in all age groups, except for an opposite tendency in the youngest age group of 16–25 years (Figure 2).

By plotting the frequency of anti-HEV positivity by birth decade, a significant decrease in anti-HEV IgG positivity was found for HIV-infected patients born in later birth decades ($p < 0.00001$) (Figure 3). With very few individuals in the cohorts being born in the 1910s ($n = 2$) and the 1990s ($n = 12$) and none of them being anti-HEV-positive, no data are visualized in the figure for these birth decades.

Discussion

In this study, a high but overall stable prevalence rate of anti-HEV IgG by time close to enrolment was found in two cohorts of HIV-infected patients, with rates of 23.3%, 22.8%, and 23.8% over three decades from the early 1980s, the 1990s, and 2000–2013, respectively.

In all three decades, the HEV seroprevalence rate increased with increasing age and the odds of being anti-HEV-positive were higher in older generations compared to individuals in the younger birth cohorts. MSM had lower odds of being anti-HEV-positive compared to HSX. Additionally, no persistent HEV infections were found in serial samples from anti-HEV seroconverting patients living with HIV.

HEV seroprevalence rates among HIV-infected individuals vary widely between geographical areas, from 2.9% in Switzerland to 37.8% in France (Debes et al., 2016; Abravanel et al., 2017). A comparison of seroprevalence studies using different anti-HEV IgG assays should be interpreted with caution because the sensitivity and specificity of anti-HEV assays vary considerably. The assay from Wantai used in the current study was selected because studies have shown it to have higher sensitivity and specificity than other assays, even when testing immunocompromised patients (Abravanel et al., 2013; Pas et al., 2013).

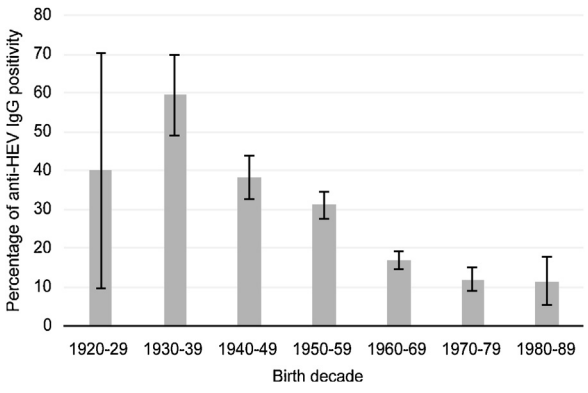

Figure 3. Frequency (%) of anti-hepatitis E virus immunoglobulin G positivity by birth decade.

The increasing presence of anti-HEV IgG with age can be explained by the cumulative exposure to HEV over time, as foodborne exposure is supposedly the main transmission route of HEV, whether HIV-infected or not.

A study of anti-HEV seroprevalence among healthy blood donors in Denmark in 2013 that also used the Wantai assay, showed a similar high rate and increase with age of anti-HEV, with a prevalence rate of 19.8% among the blood donors (Holm et al., 2015). This is comparable to the result of 23.8% among HIV-infected individuals in the decade spanning 2000–2013 in the present study. This finding of similar HEV seroprevalence among HIV-infected patients and blood donors is in concordance with case–control studies showing that the HEV seroprevalence rate among HIV-infected individuals is not higher than the rate among non-HIV-infected individuals. Keane et al. found an HEV seroprevalence of 9.4% in the HIV-infected population, which was not significantly different from age and sex-matched controls of non-HIV-infected patients, in whom the HEV seroprevalence rate was 13.8% (Keane et al., 2012). Abravanel et al. (2017) even found a significantly lower level of HEV seroprevalence among HIV-infected persons (38.7%) in France, when compared to age and sex-matched blood donors (47.3%) from the same geographical area (Abravanel et al., 2017).

Although the HEV seroprevalence rates in the current study did not, in general, decline over the decades, as shown in previous studies of Danish blood donors, a decreasing tendency of anti-HEV positivity was found in most age groups of HIV-infected individuals.

HEV seroprevalence studies among healthy Danish blood donors from 1983, 2003, and 2013 showed decreasing seroprevalence rates of 32.9%, 20%, and 10.7%, respectively, when using the same anti-HEV IgG assay from the National Institutes of Health, USA (Christensen et al., 2008; Holm et al., 2015).

In the blood donor study by Christensen et al., a cohort effect could explain the decreasing results of HEV seroprevalence rates, with donors born after 1945 having a lower degree of exposure to HEV compared to donors born before 1945. This was also reflected among the HIV-infected individuals in Denmark in the current study, where the frequency of anti-HEV positivity was significantly higher among older generations compared with generations born later. This result could also indicate a cohort effect, where earlier generations had been more exposed to HEV than later generations.

In the assessment of factors associated with the presence of anti-HEV IgG, older generation, HIV transmission route, and country of origin were significantly associated with anti-HEV IgG at baseline in multivariable logistic regression analyses of the prospective HIV cohort.

Individuals of Asian descent showed a higher likelihood of anti-HEV IgG positivity compared to those of European descent. This could be due to exposure to both the zoonotic and the obligate human HEV genotypes (gt1 and gt2), as the latter are more widespread in Asia (Van der Poel, 2014).

MSM have a higher risk of viral hepatitis infections including hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C virus (HCV) through sexual exposure (Kahn, 2002). In the present study, individuals who reported MSM as a likely source of HIV transmission were less likely to be anti-HEV-positive. The results indicate that HEV is not primarily sexually transmitted and contradict other studies that have shown MSM to be at increased risk of anti-HEV positivity regardless of HIV status, which was believed to be due to sexual practices with faecal–oral

transmission (Payne et al., 2013; Montella et al., 1994). Results from other association studies on HIV infection risk factors and HEV have previously found no association between HIV risk groups and HEV (Kenfak-Foguena et al., 2011; Pineda et al., 2014).

The zoonotic HEV genotypes – especially HEV-gt3 – are endemic in industrialized countries such as Denmark. The finding of high HEV seroprevalence rates and few cases of clinical HEV infection suggests that subclinical and self-limiting infections are common. HEV-gt3 may cause chronic or persistent infections among immune deficient patients, including cases described in HIV-infected individuals (Dalton et al., 2009; Colson et al., 2011). In the current study, no sign of persistent HEV infection was found among individuals for whom a time of seroconversion could be estimated. Of note, HEV RNA was only detected in a single sample from 276 specimens serially sampled around the time of seroconversion in 57 individuals. Of these, 37 individuals were treated in the era of antiretrovirals and 20 individuals were not treated and were therefore presumably more profoundly immune deficient.

The study findings confirm that persistent HEV infection is a rare event among HIV-infected patients. In a Spanish prospective study of 849 HIV-infected individuals, no chronic HEV infections were detected despite transient detection of HEV RNA among 5.7% of the investigated population and an anti-HEV prevalence of 9.8% (Rivero-Juarez et al., 2015). A retrospective study in the USA among 194 HIV-infected military beneficiaries with acute liver enzyme elevation (between 1985–2009) found no patients with persistent HEV viremia, and only one patient with transient HEV RNA was detected (Crum-Cianflone et al., 2012).

A study among 735 HIV cohort participants in Switzerland detected prolonged HEV viremia in two HIV-infected patients with liver enzyme elevation; both patients had low CD4 counts of <150 CD4 cells/μl (Kenfak-Foguena et al., 2011).

This study has strengths and limitations. First, the study is one of the largest investigations of HEV infection in HIV-infected individuals, with more than 2500 patients examined. Furthermore, this is the first study of HEV among Danish HIV-infected patients. A limitation of the study is the risk of underestimating the HEV seroprevalence results, as it was assumed that patients with a negative anti-HEV IgG result in the most recent sample were seronegative in their first available sample by enrolment in the cohort. The period of detectable anti-HEV IgG levels after naturally acquired HEV infection is unknown, as most infections are subclinical. Su et al. described a steady decline of anti-HEV IgG levels over time and independent of the initial antibody level and predicted that 50% would be undetectable after 14.5 years (Su et al., 2017). The median follow-up interval of the anti-HEV-negative patients, i.e. from cohort entry to the latest available sample, was 7 (IQR 3–13) years. Therefore, it is possible that some individuals may have had anti-HEV IgG levels that declined below the limit of detection over time. However, using the S/CO ratio as a measurement of the anti-HEV titre showed a modest decline in the S/CO ratio over 8 years, supporting the assumption that the earliest available test likely would be negative if the latest available test was negative.

Another limitation is the risk of underestimating persistent HEV infections due to the testing strategy for detecting persistent viremia among patients who eventually seroconverted to anti-HEV IgG. This strategy could have excluded patients with a poor immunological status, who are not able to produce detectable HEV antibodies. Thus, with the testing strategy used, the rate of persistent infections could only be detected among the HIV-infected individuals who had anti-HEV seroconverted.

In conclusion, this study found a high HEV seroprevalence rate of 23% among newly enrolled HIV-infected individuals in two Danish cohorts from the early 1980s to 2013. The HEV seroprevalence rate increased with older age, and anti-HEV positivity was associated with birth cohort, HIV risk group, and geographical origin. No evidence of persistent HEV infection in HIV-infected individuals was found, either before or after the era of effective antiretroviral treatment for HIV.

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Grifols Diagnostic Division (Emeryville, CA, USA) donated HEV RNA assays for the investigation.

Ethical approval

The study was approved by the local ethics committee for the Capital Region of Denmark (No. H-18045809) and the Danish Data Protection Agency (No. 2007-41-1634).

Conflict of interest

No conflicts of interest exist for any of the authors regarding this manuscript.

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