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Acute and Chronic Hepatitis E Virus Infection in HIV-Infected United States Women

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Abstract

Exposure to hepatitis E virus (HEV) is common in the United States (US) but there are few data on prevalence of HEV/HIV co-infection in US populations. We tested 2,919 plasma samples collected from HIV-infected (HIV^+) women and men enrolled in US cohort studies for HEV viremia using a high-throughput nucleic acid testing (NAT) platform. NAT^+ samples were confirmed by real-time polymerase chain reaction (PCR). Samples were selected for testing primarily on the basis of biomarkers of liver disease and immune suppression. Prevalence of HEV viremia was 3/2,606 and 0/313 in tested plasma samples collected from HIV^+ women and men, respectively. All HEV isolates were genotype 3a. Based on follow-up testing of stored samples, one woman had chronic HEV infection for >4 years while 2 women had acute HEV detectable at only a single study visit.

Conclusion—To our knowledge this is the first reported case of chronic HEV infection in an HIV^+ US individual. We also confirm that chronic HEV infection can persist despite a CD4+ count >200 cells/mm^3. These data suggest that HEV infection is rare in the HIV^+ US population.

Keywords

HEV; Human immunodeficiency virus; RNA; PCR; ALT

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CONFLICTS OF INTEREST

Edgar Ong and Jeffrey M. Linnen are employees of Hologic, Inc. which in partnership with Grifols Diagnostic Solutions (Emeryville, CA), developed the Procleix HEV assay. All other authors, no conflicts of interest declared.

INTRODUCTION

Hepatitis E virus (HEV) is a single-stranded non-enveloped RNA virus in the family Hepeviridae.(1) Only a single HEV serotype has been identified but four HEV genotypes circulate in humans.(1;2) HEV genotypes 1 and 2 are transmitted by the fecal-oral route and are responsible for sporadic cases and large outbreaks of acute hepatitis among people living in or traveling to developing countries. Together HEV genotypes 1 and 2 are estimated to cause 3.4 million cases of acute hepatitis and 70,000 deaths annually.(3)

Unlike HEV genotypes 1 and 2 which infect only humans, HEV genotypes 3 and 4 are zoonotic and have been detected in humans with sporadic acute hepatitis in both developing and industrialized countries as well as in pigs, wild boar and deer.(1) Direct contact with or consumption of pigs, wild boar and deer (and possibly other mammals), exposure to pig/boar/deer or human sewage and blood transfusion are risk factors for HEV genotype 3 and 4 infections.(4;5) These risk factors are, however, not reported by some people with HEV genotype 3- or 4-associated acute hepatitis(6;7) and thus the epidemiology of these genotypes is incompletely understood. HEV genotype 3 has been detected in humans and pigs/boars worldwide including in the United States (US).(8;9) HEV genotype 4 is most often detected in humans and pigs/boars/deer in East Asia but has also been detected in Europe.(1)

Lifetime exposure to HEV – as indicated by the presence of HEV immunoglobulin (Ig) G in serum or plasma – is common in the US and many European countries although estimates of HEV IgG seroprevalence vary widely (e.g., 6%-53%) and depend on the choice of HEV serologic assay, the age of the study population and the geographic region.(10–14) It is believed that most HEV genotype 3 and 4 infections in humans are asymptomatic, with only a small fraction of infections manifesting as acute hepatitis.(15) However, in some European countries hundreds of HEV genotype 3-associated acute hepatitis cases are identified annually.(16;17) In the US, in contrast, HEV genotype 3-associated acute hepatitis is thought to be rare with only 15 probable cases identified by the US Centers for Disease Control and Prevention (CDC) during surveillance from 2005–2012.(9)

To better understand the epidemiology and clinical importance of HEV in the US, we tested 2,919 plasma samples collected from HIV-infected (HIV^+) women and men enrolled in two multi-site prospective US cohort studies for HEV viremia using a high-throughput nucleic acid testing (NAT) platform. Prior studies of US and European populations have not found an association between HIV status and HEV seroprevalence,(14;18) but we hypothesized that HEV testing of participants in HIV cohorts would be particularly informative because: (i) blood samples and detailed clinical information are collected from participants longitudinally on a semi-annual basis, (ii) immune suppression, including HIV-associated immune suppression, is a risk factor for chronic HEV infection and rapidly progressing liver disease(19) and (iii) pre-existing liver disease – which in some small studies has been associated with severe HEV-associated hepatitis and death(20;21) – is common in these cohorts because of high prevalence of hepatitis C virus (HCV) and hepatitis B virus (HBV) co-infections.

METHODS

Study Population

The study population included subsets of the HIV$^+$ women enrolled in the Women's Interagency HIV Study (WIHS) and the HIV$^+$ men enrolled in the Multicenter AIDS Cohort Study (MACS). Detailed methods and characteristics of the WIHS(22) and MACS(23) populations have been described previously. Briefly, the WIHS is a prospective study of HIV$^+$ and HIV$^-$ women who were recruited using similar methods at six US sites (Bronx, New York; Brooklyn, New York; Washington, District of Columbia; Chicago, Illinois; San Francisco, California; Los Angeles, California) during two recruitment periods: 1994–1995 and 2001–2002. The MACS is a prospective study of HIV$^+$ and HIV$^-$ men who have sex with men (MSM) at four US sites (Baltimore, Maryland/Washington, District of Columbia; Chicago, Illinois; Pittsburgh, Pennsylvania; and Los Angeles, California) enrolled during three recruitment periods: 1984–1985, 1987–1990, and 2001–2003. All WIHS and MACS participants provided informed consent, and the institutional review boards (IRBs) at each site approved the study. This nested HEV substudy was approved by the IRB of the Albert Einstein College of Medicine.

Inclusion criteria for this study are shown in Table 1. Samples were selected for testing primarily on the basis of biomarkers of liver disease and immune suppression because it was hypothesized that these characteristics are associated with prevalence of HEV viremia (i.e., ↓ immune status = ↑ HEV prevalence; ↑ liver disease = ↑ HEV prevalence). Specifically, for HIV$^+$ WIHS women, we tested reposited plasma stored at −80°C from study visits by participants representing several levels of liver disease severity based on levels of alanine aminotransferase (ALT), aspartate-to-platelet ratio (APRI) and FIB-4 (groups 1–5 – APRI and FIB-4 were calculated based on published equations (24;25)), immune suppression based on CD4+ T-cell counts (groups 6–7) and controls without evidence of liver disease or immune suppression (group 8). For HIV$^+$ MACS men, we tested plasma from: (group 1) men who died of liver disease – the last person-visit prior to death and, if available, from an earlier visit with the lowest CD4+ count and (group 2) up to two person-visits with ALT ≥100 in men who were HCV RNA$^-$ and HBsAg$^-$. WIHS plasma samples were never thawed prior to shipment to the HEV testing laboratories at −20°C. Among the MACS samples, 276 were never thawed, 36 had been thawed once and 1 sample was thawed twice prior to shipment.

Laboratory Methods

HEV viremia screening test—One 0.5 mL plasma aliquot for each of the 2,919 HIV$^+$ study visits was tested with the Procleix HEV assay on the Procleix Panther system (Hologic, Inc., San Diego, CA/Grifols Diagnostic Solutions, Inc., Emeryville, CA) at Hologic facilities in San Diego (Figure 1). The Procleix HEV assay is based on a transcription-mediated amplification (TMA) assay and performed on the Panther system, a fully automated platform. Analytical sensitivity panels composed of serially diluted HEV World Health Organization (WHO) International Standard (PEI Code 6329/10) and a panel of *in vitro* synthesized transcripts corresponding to HEV genotypes and subtypes were used to evaluate assay sensitivity. The panels were tested with the HEV assay on the Panther

system. The TMA assay showed a 95% limit of detection (LOD) of 7.9 IU/mL using the WHO standard.(26) The assay detected all four major HEV genotypes (1, 2, 3, and 4) with 95% LOD values ranging from 7.9 to 17.7 copies/mL using RNA transcripts for HEV 1, 2, 3a, 3b, 3f, and 4c.(26)

In this study, the plasma samples were tested individually on the Panther system. Initial TMA-reactive samples were retested if sufficient volume of sample remained, and a sample was considered positive if the retest result was reactive. Following identification of TMA-reactive samples, we requested repository plasma from all study visits through October 2013 for screening test-positive participants (Figure 1). These additional plasma samples were also tested with the Procleix HEV assay as well as with an HEV RNA confirmatory NAT and for HEV antibodies (see below). Plasma samples through April 2015 were subsequently requested for a participant with chronic HEV.

HEV viremia confirmatory test—For confirmation of TMA-reactive study visits and for testing of plasma from all study visits for screening test-positive participants, plasma aliquots were tested at Sanquin Diagnostics (Amsterdam, the Netherlands) by amplification of a 74-bp fragment of HEV open reading frame (ORF) 3.(27) RNA was extracted from 0.4 mL of plasma using the QIAamp MinElute virus spin kit (Qiagen, KJ Venlo, the Netherlands) and eluted in 50 μL according to the manufacturer's protocol. MS2 phage was added before extraction as an internal control. Amplification used 20 μL of eluate in a 50-μL volume using TaqMan Fast Virus one-step master mix (TaqMan Fast, Life Technologies, Carlsbad, CA). Polymerase chain reaction (PCR) was performed using a real-time PCR system (LightCycler 480-II, Roche, Basel, Switzerland) and standard PCR conditions. Reactions were performed in duplicate, with and without MS2 detection using MS2-specific primers and a HEX/BHQ1-labeled TaqMan probe.(27) The test has a 95% LOD of 10.3 IU/mL based on the WHO standard. If duplicates were discrepant the test was labeled inconclusive or borderline depending on the robustness of the fluorescent signal.

HEV genotype sequencing—HEV genotyping was performed by PCR amplification of a 686-bp fragment of the ORF2 region(28) using AMV RT and GoTaq DNA polymerase (Promega, Madison, WI) according to the manufacturer's instructions. If no PCR product was detected a 326-bp fragment was amplified using nested primers.(28) PCR fragments were sequenced using an ABI Prism 3130xl genetic analyser (Thermo Fisher Scientific, Waltham, MA) according to standard protocol. Sequence analysis was performed with computer software (Lasergene, DNASTAR, Madison, WI and Geneious, Biomatters, Auckland, New Zealand), using HEV reference sequences as described by Smith and colleagues(29) and additional HEV sequences from GenBank. Genetic distances were calculated using the Tamura-Nei model; the phylogenetic tree was constructed using the neighbor-joining method.

HEV IgM and IgG antibody testing—We tested TMA-reactive samples and plasma from all person-visits for screening test-positive participants for HEV IgG and IgM antibodies using the Wantai IgG and IgM enzyme-linked immunosorbent assay (ELISA) tests (Beijing Wantai Biological Pharmacy Enterprise Co., Beijing, China), according to the manufacturers' instructions (Figure 1).

Clinical laboratory measurements—ALT, aspartate aminotransferase (AST) and platelets were measured in Clinical Laboratory Improvement Amendments (CLIA)-approved laboratories at each WIHS study site. Total CD4+ T-cell counts (cells/mm^3) were determined by flow cytometry in laboratories participating in the DAIDS Quality Assurance Program at each WIHS study site.(30) Protocols for HCV and HBsAg testing in WIHS and MACS have been described previously.(31–34)

Statistical Analysis

Exact confidence intervals for one proportion were calculated using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Characteristics of the tested plasma samples

Tested in this study were plasma samples collected at 2,606 WIHS and 313 MACS person-visits (from 1,607 HIV^+ women and 190 HIV^+ men, respectively), selected according to the inclusion criteria shown in Table 1. The number of plasma samples per WIHS woman and MACS man was: 1 sample, 895 women and 70 men; 2 samples, 499 women and 118 men; 3 samples, 167 women and 1 man; 4 samples, 32 women and 1 man; 5 samples, 9 women; 6 samples, 3 women; 10 samples, 1 woman; 11 samples, 1 woman. Plasma samples collected from April 1984 through March 2013 were tested in this study – in WIHS the median (interquartile range (IQR)) sample collection date was June 14, 2001 (July 24, 1996–Jan 17, 2006) and in MACS the median (IQR) collection date was May 20, 2003 (October 3, 1991–July 14, 2007).

Characteristics of the study participants

Selected characteristics at enrollment into WIHS and MACS for the 1,607 women and 190 men are shown in Table 2.

HEV viremia in the tested plasma samples

Four plasma samples collected from four different WIHS women were reactive on the HEV screening test (Procleix HEV assay). For three of these samples, a retest using the TMA assay also yielded reactive results (Supplementary Table 1). For the fourth sample there was insufficient volume of plasma remaining for a TMA retest, thus, TMA retesting was not conducted. No plasma samples collected from MACS men were reactive on the HEV screening test.

The three retested TMA-reactive plasma samples were also reactive on the HEV confirmatory PCR test while the sample with too little volume for TMA retesting was negative on the confirmatory PCR test (Supplementary Table 1). Point prevalence of confirmed HEV viremia in the selected WIHS samples is therefore 3/2,606 or 0.0012 (exact 95% confidence interval (CI): 0.0002, 0.0034), or ~1 per 1,000 samples.

Follow-up testing

Plasma from all WIHS study visits through October 2013 for women with a confirmed HEV viremic study visit were tested subsequently with the TMA assay, the real-time PCR assay and for HEV IgG and IgM antibodies with the Wantai assay. One woman (Study ID #1) had chronic HEV viremia starting in 2010 and persisting through 2013 while the other two women (Study IDs #2 & #3) had HEV viremia detected at only a single WIHS study visit (Supplementary Table 1). Selected characteristics of the three WIHS women with confirmed HEV viremia are shown in Table 3. We subsequently tested the most recent study visits for Study ID #1 – she remained HEV viremic through April 2015 (Supplementary Table 1).

Anti-HEV IgG testing revealed that the two women with HEV viremia detected at only a single WIHS study visit (Study IDs #2 and #3) followed a classical seroconversion pattern. That is, anti-HEV IgG was first detectable at or immediately following the study visit at which HEV viremia was detected. Anti-HEV IgM was not, however, detected in these women at any study visit (Supplementary Table 1). In contrast, no clear anti-HEV IgG seroconversion pattern was observed for the woman with chronic HEV viremia (Study ID #1), albeit a consistent seroconversion appears to have commenced in 2014, >3 years after HEV viremia was first detected in this woman. The anti-HEV IgM pattern for Study ID #1 partially overlapped with her anti-HEV IgG pattern, with IgM detectable in all samples collected in 2014–2015 (Supplementary Table 1).

Figure 2 panels A–C show patterns of ALT, AST, CD4+ count, HEV viremia and antibodies over time through 2013 for the three women with confirmed HEV viremia. As seen in panel A, the woman with chronic HEV (Study ID #1) was immune suppressed (i.e., CD4+ count <200 cells/mm^3) at most study visits prior to HEV infection. However, she remained persistently HEV viremic despite a study visit where CD4+ count was >200 cells/mm^3. ALT and AST levels for Study ID #1 were highly elevated (i.e., >100 IU/L) at one visit ~2 years after the initial HEV viremic study visit. For Study ID #2 (Figure 2, panel B), the study visit at which HEV viremia was detected was characterized by highly elevated ALT. For Study ID #3 (Figure 2, panel C), her HEV viremic study visit was characterized by highly elevated AST but not ALT. Neither Study IDs #2 or #3 were immune suppressed at their HEV viremic study visit.

Phylogenetic analysis

Amplification and sequencing of a 686 bp HEV ORF2 fragment was performed using plasma from all confirmed positive participants; two samples (collected in 2012 and 2014) were analyzed from the chronically infected participant. In one case (Study ID #3) no PCR fragment was detected but a 304 bp nested fragment was successfully amplified. Phylogenetic analysis of the HEV sequences (Supplementary Figure 1) showed all sequences were most homologous to genotype 3a sequences (which are part of the 3abchij clade).(29) A 582 bp fragment from the chronically infected participant was identical in 2012 and 2014 (except for one ambiguous base; the remaining part of the 2014 sequence contained too much noise to interpret). Genbank accession numbers are KT718045, KT718043 and KT718044 for sequences amplified from Study IDs #1, #2, and #3, respectively.

DISCUSSION

Herein we report for the first time a case of chronic HEV infection in an HIV$^+$ US person. We also identified 2 cases of acute HEV viremia in HIV$^+$ US people. Prevalence of HEV viremia was 3/2,606 and 0/313 in tested plasma samples collected from HIV$^+$ women and men, respectively. All HEV isolates were genotype 3a. Taken together, these data suggest that HEV infection is rare in the HIV$^+$ US population.

HEV viremia was also rare in prior studies of HIV$^+$ populations in industrialized countries. Several moderately sized (n=93–231) European(18;35–38) and US(39) studies did not detect any HEV viremia in HIV$^+$ patient populations. Other moderately sized (n=115–735) European(40–43) and US(44) studies detected HEV viremia in 1 or 2 patients. The current investigation was ≥4 times larger than prior studies so identification of more participant samples with prevalent HEV viremia (3 vs. 2, 1 or 0 in prior studies) is not surprising even if actual HEV incidence rates vary by geography and/or other factors.

The current study avoided testing algorithms based on HEV serology. In some prior studies every HIV$^+$ patient sample was tested for HEV RNA(18;35–37;43) but in most studies HEV RNA testing was limited to samples with specific serologic profiles (e.g., HEV IgM+ and/or HEV IgG+). Testing algorithms based on HEV serology may, however, be problematic because appearance of HEV IgG/IgM antibodies following HEV infection can be delayed or absent in immune suppressed individuals, including in HIV$^+$ individuals.(40;45). Another well recognized concern is that sensitivity and specificity of HEV serologic assays vary widely.(46;47) Although the HEV serologic assays used in the current study (Wantai) are estimated to be among the more sensitive and specific of HEV serologic assays,(46;47) a testing algorithm based on Wantai HEV IgM reactivity would have missed all 3 cases of prevalent HEV viremia in WIHS women. In contrast, Wantai HEV IgG seroconversion was clearly observed for 2 women. Wantai HEV IgG seroconversion was delayed for the woman with chronic HEV (Supplementary Table 1).

Only one prior observation of chronic HEV infection has to our knowledge been reported in the US, in a Pennsylvania renal transplant recipient.(48) The current study adds to these prior data in several ways: (i) we show that HIV$^+$ US populations are also at risk for chronic HEV; (ii) we confirm the findings of prior reports(49–51) in which chronic HEV infection can persist despite a CD4+ T-cell count >200 cells cells/mm^3 (Figure 2, Panel A) and (iii) we observe that chronic HEV can persist for 2 years without ALT/AST elevation (Figure 2, Panel A).

HEV diagnostic testing is not routinely available in the US. Health care providers, public health departments, and diagnostic laboratories may send serum/stool samples to the US CDC for HEV testing(9) but it is unclear whether more systematic testing of US populations, including HIV$^+$ populations, will lead to reductions in HEV-associated morbidity and mortality or just uncover asymptomatic genotype 3 infections. In accordance with this study's approved IRB protocol, Study ID #1 is currently being informed of her chronic HEV viremia status. HEV Study ID #2 died of liver-related causes in 2010 but it is unclear if her acute HEV infection in 1998 contributed to her death – she had chronic HCV

infection and reported consumption of alcohol at enrollment into WIHS in 1995 (Table 3) and at the majority of her follow-up visits (data not shown).

Several facts are important to consider in the interpretation of these data. First, the prevalence estimates of HEV viremia are in those samples selected for HEV screening according to the inclusion criteria (Table 1). Second, WIHS and MACS are HIV-focused cohorts and have not collected behavioral information that may be particularly informative for understanding routes of HEV acquisition (e.g., travel history, contact with or consumption of pigs/boar, sources of drinking water). Third, there is a lag period between WIHS study visits and release of data to WIHS investigators – the most recent (2014–2015) CD4+/ALT/AST data for HEV Study ID #3 were not available at the time of this writing. Fourth, lack of HCV RNA and HBsAg testing data at every WIHS visit limits the precision of results related to these infections including our ability to measure successful treatment and spontaneous clearance over time. Fifth, lack of HEV IgG and IgM data for all 2,919 samples screened for HEV RNA is a limitation – however, as mentioned above appearance of HEV IgG/IgM antibodies following HEV infection can be delayed or absent in immune suppressed individuals and a well recognized concern is that sensitivity and specificity of HEV serologic assays vary widely.(46;47) Sixth, inclusion criteria for selection of WIHS and MACS samples for HEV RNA screening were not uniform - these two study populations differed in meaningful ways (e.g., CD4+ T-cell counts at enrollment were higher in MACS men vs. WIHS women).

In conclusion, this study provides evidence that acute and chronic HEV infections occur in the HIV^+ US population. HEV viremia was detected in ~1 per 1,000 samples tested primarily on the basis of biomarkers of liver disease and immune suppression. Although recent European case reports show positive HIV^+ patient outcomes following detection of chronic HEV and administration HEV antiviral therapy,(49;52) the rarity of HEV viremia in the WIHS/MACS study populations suggests that widespread screening for HEV in HIV^+ US populations is not warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LIST OF ABBREVIATIONS

HEV	hepatitis E virus
US	United States
NAT	nucleic acid testing
PCR	polymerase chain reaction
Ig	immunoglobulin
CDC	Centers for Disease Control and Prevention
HCV	hepatitis C virus
HBV	hepatitis B virus
WIHS	Women's Interagency HIV Study
MACS	Multicenter AIDS Cohort Study
MSM	men who have sex with men
IRB	institutional review board
ALT	alanine aminotransferase
APRI	aspartate-to-platelet ratio
TMA	transcription-mediated amplification
WHO	World Health Organization
LOD	limit of detection
ORF	open reading frame
ELISA	enzyme-linked immunosorbent assay
AST	aspartate aminotransferase

CLIA	Clinical Laboratory Improvement Amendments
IQR	interquartile range

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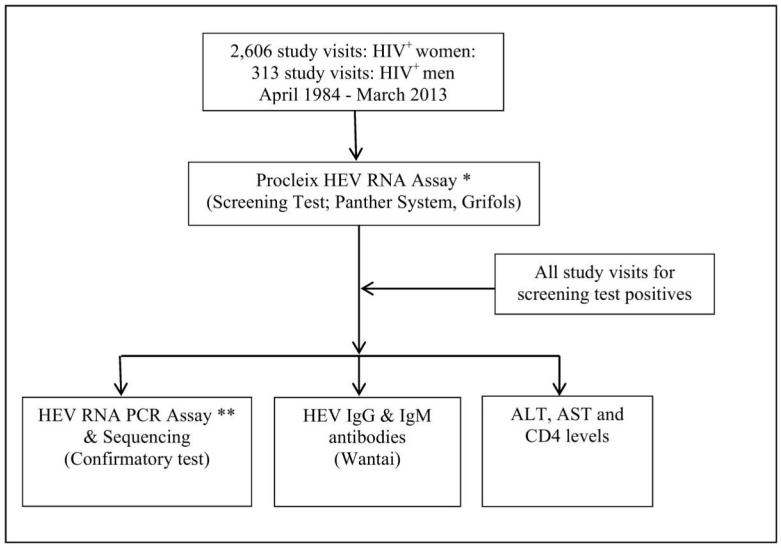

Figure 1.
HEV study design. *TMA assay; **in-house real-time PCR

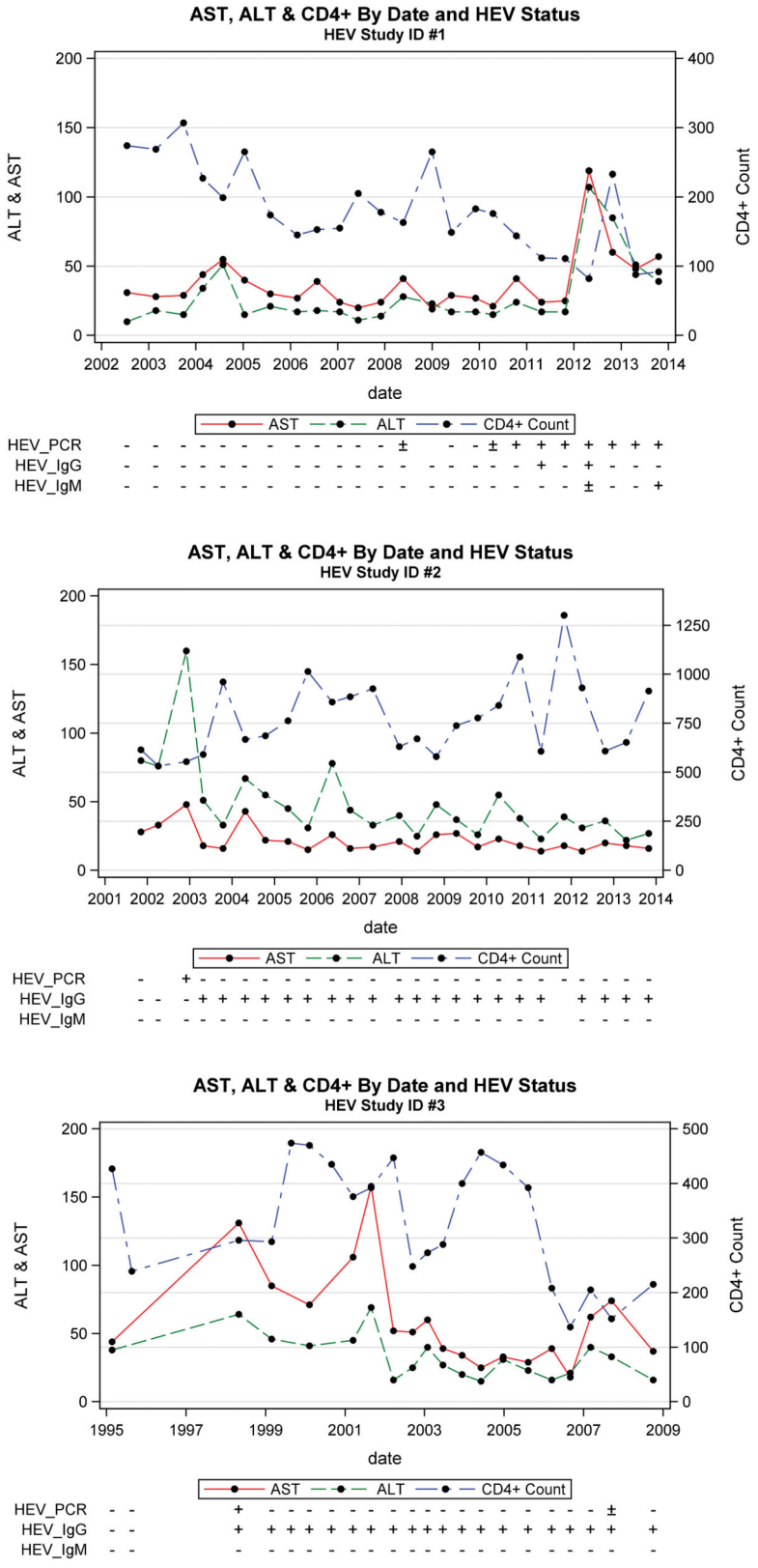

Figure 2.

Panels A–C. Patterns of ALT, AST, CD4+ count, HEV viremia and antibodies over time through 2013 for three women with confirmed HEV viremia (Panels A–C). ALT and AST are in IU/L and CD4+ counts are cells/mm^3. The ± symbol represents an inconclusive or borderline test result. ALT and AST measurements in WIHS were conducted on an annual basis (every other WIHS visit) from 1994–2001 and on a semi-annual basis (every WIHS visit) thereafter. CD4+ measurements in WIHS are conducted on a semi-annual basis (every WIHS visit).

Table 1

Inclusion criteria for HIV$^+$ women and men

Group	Selection criteria #1		#2		#3		#4	# visits requested from repository	# visits available for testing
WIHS women[b]									
1	ALT >100	AND	HCV RNA(−)	AND	HBsAg(−)			500	248
2	ALT >100	AND	HCV RNA(+)	OR	HBsAg(+)			250	218
3	50≤ ALT ≤100	AND	HCV RNA(−)	AND	HBsAg(−)			250	250
4	APRI >1.5	AND	FIB-4 >3.25	AND	HCV RNA(−)	AND	HBsAg(−)	250	164
5	APRI >1.5	AND	FIB-4 >3.25	AND	HCV RNA(+)	OR	HBsAg(+)	250	249
6	CD4 <50							1000	976
7	50≤ CD4 ≤200							250	251
8	CD4 >200	AND	ALT <50	AND	APRI ≤0.5	AND	FIB-4 <1.45	250	250
MACS men[b]									
1	Liver death							NA	153
2	ALT ≥100	AND	HCV RNA(−)	AND	HBsAg(−)			NA	160

ALT: alanine aminotransferase; HCV: hepatitis C virus; HBsAg: Hepatitis B surface antigen; CD4: cluster of differentiation 4; APRI: AST to Platelet Ratio; NA: Not applicable

[a] ALT levels are in IU/L and CD4 levels are cells/mm^3

[b] The sample selection process differed for WIHS and MACS. For WIHS, we chose selection criteria and a maximum number of samples to test and then queried the WIHS repository to assess the number available for testing. If excess samples were available that met specific selection criteria, a random subset was selected. For MACS, the selection process excluded visits that had been found to have limited availability during earlier unrelated hepatitis testing, and we also excluded from consideration any visits that had previously been restricted by the MACS executive committee (EC).

Table 2

Selected characteristics of HIV$^+$ study participants at enrollment into the WIHS and MACS cohorts

	WIHS (n=1607)	MACS (n=190)
	N (%)	**N (%)**
Race/ethnicity		
White	120 (7%)	129 (68%)
African American	1019 (63%)	24 (13%)
Hispanic	429 (27%)	32 (17%)
Other	39 (2%)	5 (3%)
HCV RNA+	379 (24%)[a]	17 (9%)[a]
HBsAg+	37 (2%)[b]	36 (19%)
Alcohol >12 drinks/wk	131 (8%) [b]	43 (23%)
	Median (IQR)	Median (IQR)
Enrollment date	04/1995 (02/1995, 02/2002)	12/1984 (08/1984, 05/2002)
Age	36 (30, 41)	33 (27, 39)
CD4+ count[c]	288 (122, 462)	564 (420, 727)

WIHS: Women's Interagency HIV Study; MACS: Multicenter AIDS Cohort Study; HCV: hepatitis C virus; HbsAg: Hepatitis B surface antigen; CD4: cluster of differentiation 4; IQR: interquartile range

[a] HCV RNA was only assessed among HCV antibody-positive participants

[b] % among participants with available data

[c] CD4+ counts are in cells/mm^3

Table 3

Selected characteristics of HIV$^+$ WIHS women with confirmed HEV viremia

	HEV Study ID		
	1	**2**	**3**
Inclusion criteria group[a]	1	1	5
Age at enrollment	37	33	41
Date of enrollment	07/2002	11/2001	02/1995
Race/ethnicity	African American	Hispanic	White
Study site	Brooklyn	San Francisco	Chicago
Date of HEV infection	04/2010	12/2002	04/1998
Chronic HEV	+	−	−
Date of HAART initiation	03/2001	08/1998	04/1998
Date of death	NA	NA	09/2010
Cause of death	NA	NA	Non-AIDS, liver
Alcohol use at enrollment	Abstainer	Abstainer	>12 drinks/wk
HCV RNA at enrollment	−	−	+
HBsAg at enrollment	−	−	−

WIHS: Women's Interagency HIV Study; HCV: hepatitis C virus; HbsAg: Hepatitis B surface antigen; HAART: highly active antiretroviral therapy; NA: not applicable

[a] See Table 1 for a definitions of inclusion criteria groups