Hepatitis C Virus Reinfection and Spontaneous Clearance of Reinfection—the InC^3 Study

Rachel Sacks-Davis,[1,2,a] Jason Grebely,[3] Gregory J. Dore,[3] William Osburn,[5] Andrea L. Cox,[5] Thomas M. Rice,[6] Timothy Spelman,[1,2] Julie Bruneau,[10] Maria Prins,[11,12] Arthur Y. Kim,[7] Barbara H. McGovern,[8,9] Naglaa H. Shoukry,[10] Janke Schinkel,[12] Todd M. Allen,[7] Meghan Morris,[6] Behzad Hajarizadeh,[3] Lisa Maher,[3] Andrew R. Lloyd,[4] Kimberly Page,[6,a] and Margaret Hellard[1,2]; on behalf of the InC^3 study group

[1]Burnet Institute, [2]Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, [3]The Kirby Institute, and [4]School of Medical Sciences, University of New South Wales, Sydney, Australia; [5]Department of Medicine, Johns Hopkins University, Baltimore, Maryland; [6]Department of Epidemiology and Biostatistics, University of California–San Francisco; [7]Harvard Medical School, [8]Tufts Medical School, Boston, Massachusetts; [9]Abbvie, Chicago, Illinois; [10]CRCHUM, Université de Montréal, Canada; [11]GGD Public Health Service of Amsterdam, and [12]Academic Medical Center, Amsterdam, The Netherlands

Background. We aimed to characterize the natural history of hepatitis C virus (HCV) reinfection and spontaneous clearance following reinfection (reclearance), including predictors of HCV reclearance.

Methods. Data were synthesized from the 9 prospective cohorts of the International Collaboration of Incident Human Immunodeficiency Virus and HCV in Injecting Cohorts study, which evaluated HCV infection outcomes among people who inject drugs. Participants with primary HCV infection were classified as having achieved viral suppression if they had negative results of at least 1 subsequent HCV RNA test. Those with positive results of an HCV RNA test following viral suppression were investigated for reinfection. Viral sequence analysis was used to identify reinfection (defined as detection of heterologous virus with no subsequent detection of the original viral strain).

Results. Among 591 participants with acute primary HCV infection, 118 were investigated for reinfection. Twenty-eight participants were reinfected (12.3 cases/100 person-years; 95% confidence interval [CI], 8.5–17.8). Peak HCV RNA level was lower during reinfection than primary infection ($P = .011$). The proportion of individuals with reclearance 6 months after reinfection was 52% (95% CI, 33%–73%). After adjustment for study site, females with the *IFNL4* (formerly *IFNL3* and *IL28B*) rs12979860 CC genotype detected were more likely to have reclearance (hazard ratio, 4.16; 95% CI, 1.24–13.94; $P = .021$).

Conclusions. Sex and *IFNL4* genotype are associated with spontaneous clearance after reinfection.

Keywords. hepatitis C; re-infection; viral resolution; cohort study; *IFNL4*; sex; injecting drug use.

Spontaneous clearance of primary hepatitis C virus (HCV) occurs in 25% of individuals [1]. However, reinfection following spontaneous clearance suggests that natural immunity is short-lived or has limited breadth or magnitude, with implications for vaccine development [2].

Studies of HCV reinfection in people who inject drugs have produced contradictory results [2], with variations in reinfection rates and proportions of reinfections clearing spontaneously [3–12]. These discrepancies may be attributed to methodological limitations, including variations in frequency of follow-up testing and data capture [2, 13], and to classification of viral recurrence as reinfection without confirmation that viremic episodes are genetically distinct [12].

The International Collaboration of Incident HIV and HCV in Injecting Cohorts (InC^3) study, which pooled data from 9 prospective cohorts in Australia, Canada, the Netherlands, and the United States [14], mainly comprising from people who inject drugs, enables

Received 22 September 2014; accepted 30 March 2015; electronically published 15 April 2015.

[a]Present affiliations: Department of Internal Medicine, University of New Mexico, Albuquerque (K. P.), Department of Medicine, University of Melbourne, Melbourne, Australia (R. S.-D.).

Presented in part: 20th International Symposium on Hepatitis C Virus and Related Viruses, Melbourne, Australia, 6–10 October 2013. Abstract 32434.

Correspondence: Rachel Sacks-Davis, PhD, Peter Doherty Institute for Infection and Immunity, 792 Elizabeth Street, Melbourne, VIC 3000, Australia (rachel.sacks@unimelb.edu.au).

The Journal of Infectious Diseases® 2015;212:1407–19

DOI: 10.1093/infdis/jiv220

assessment of HCV reinfection in well-characterized HCV-infected participants. The aims of our study were to characterize the natural history of spontaneous clearance following primary infection (primary clearance), HCV reinfection, and spontaneous clearance following reinfection (reclearance) in the InC[3] study; assess differences in peak HCV RNA levels during reinfection, compared with primary infection; assess predictors of HCV reclearance; and assess differences in the time to primary clearance and reclearance.

PARTICIPANTS AND METHODS

Study Population and Design

The InC[3] study has been described previously [14]. All cohorts followed up participants at regular intervals, using standardized methods (Table 1). Participants were recruited and followed up between 1985 and 2010. For the current study, only individuals with documented acute primary HCV infection and results of ≥2 subsequent HCV RNA tests were included.

Primary HCV infection is defined as an individual's first HCV infection. Documented acute primary HCV infected is defined as either (1) HCV seroconversion with an HCV antibody–negative test result followed by an HCV antibody– or HCV RNA–positive test result within 2 years or (2) evidence of symptomatic infection (defined by jaundice or an alanine transaminase level of >400 U/L, a positive HCV RNA or HCV antibody test result, and recent high-risk exposure). All participants provided written informed consent, and cohort protocols were approved by local institutional human research review committees.

Laboratory Testing

Choice of HCV RNA testing and HCV sequencing methods and regions varied between but not within cohorts. Qualitative and quantitative HCV RNA, HCV genotype and serotype, and interferon lambda 4 (*IFNL4*) rs12979860 genotype (formerly known as interferon lambda 3 and interleukin 28B) assays have been described previously [15]. Regions of the virus sequenced to confirm reinfection are listed in Supplementary Table 1. Polymerase chain reaction amplification conditions, primers, and sequencing methods have been described previously [16–20]. Cohort sites used distance [6, 7, 11] or phylogenetic methods [5] to distinguish heterologous from homologous virus within viral subtypes.

Estimated Date of Primary HCV Infection

The estimated date of primary HCV infection was determined using the flow chart in Figure 1*A*.

Antiviral Treatment for HCV Infection

The natural history of HCV reinfection and reclearance may differ following antiviral treatment, compared with spontaneous clearance of primary HCV infection. Therefore, this analysis

Table 1. Incidence Rate of Reinfection, by Cohort

Variable	Participants Evaluated, No.[a]	Reinfections, No.	Test Interval, mos	Test Interval Acute Primary HCV Infection, mos[b]	Person-Years	Rate, Cases/100 Person-Years (95% CI)
Overall	128	28	. . .	. . .	227.8	12.3 (8.5–17.8)
Cohort (location)						
ACS (the Netherlands)	13	4	6	6	67.0	6.0 (2.2–15.9)
ATAHC (Australia)	25	2	. . .[c]	3	31.0	6.5 (1.6–25.8)
BAHSTION (United States)	12	1	. . .[d]	1[e]	11.3	8.9 (1.2–62.9)
BBAASH (United States)	27	15	1	1	41.2	36.4 (21.9–60.4)
HEPCO (Canada)	7	0	6	1[f]	19.7	0.0
HITS-c (Australia)	3	0	6	0.5–1[g]	2.7	0.0
HITS-p (Australia)	18	2	6	0.5–1[g]	23.5	8.5 (2.1–34.1)
N2 (Australia)	4	2	3	3	7.4	27.1 (6.8–108.5)
UFO (United States)	19	2	3	1	24.0	8.3 (2.1–33.3)

Abbreviations: ACS, Amsterdam Cohort Studies; ATAHC, Australian Trial in Acute Hepatitis C study; BAHSTION, Boston Acute HCV Study: Transmission, Immunity, and Outcomes Network; BBAASH, Baltimore Before and After Acute Study of Hepatitis; CI, confidence interval; HCV, hepatitis C virus; HEPCO, St. Luc Cohort; HITS-c, Hepatitis C Incidence and Transmission Study–Community; HITS-p, Hepatitis C Incidence and Transmission Study–Prison; N2, Networks 2; UFO, UFO study.

[a] Includes participants with persistent spontaneous clearance and reinfection. Participants with intercalation are excluded because they are assumed not to have spontaneously cleared infection. Participants with indeterminate reoccurring viremia are excluded because the reinfection outcome is unknown.

[b] Many cohort sites perform tests more frequently after identification of acute primary HCV infection.

[c] Eligibility is based on recent HCV seroconversion or confirmed acute HCV infection.

[d] Eligibility of seronegative participants or those with acute HCV infection is based on recent exposure to HCV or suspected or acute HCV infection.

[e] For 6 months, after which the interval between tests is reduced.

[f] For 24 weeks, after which the normal test schedule resumes.

[g] For 3 months among participants with early acute infection (ie, HCV RNA is still present), after which the normal test schedule resumes.

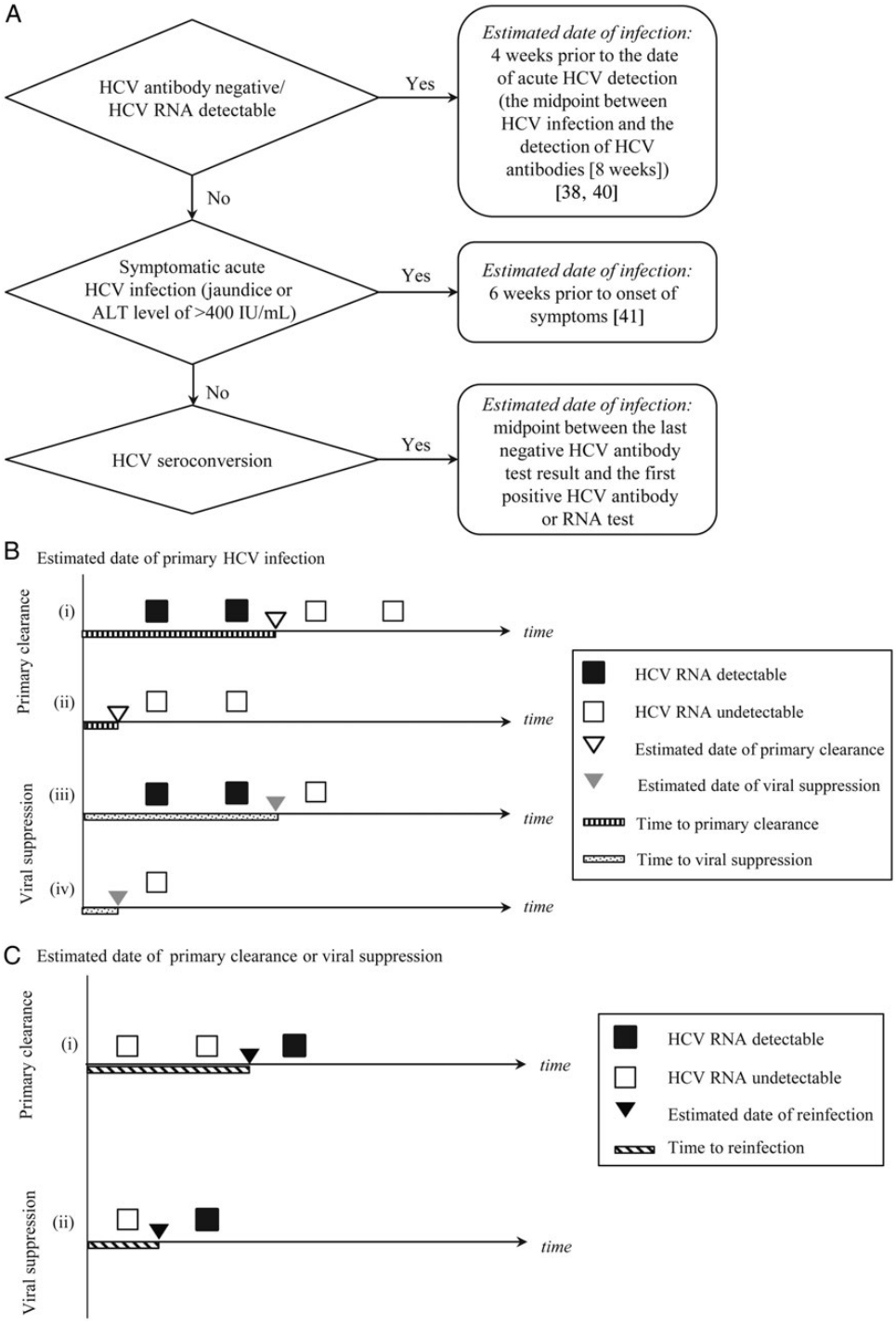

Figure 1. Timing and classification of hepatitis C virus (HCV) primary infection, viral suppression, spontaneous clearance, and reinfection. *A*, Flowchart for determining the estimated date of primary HCV infection. *B*, Estimated dates of and times to primary clearance and viral suppression. All timelines represent participants with confirmed primary HCV infection followed by either viral suppression or primary clearance. The timelines begin at the estimated date of primary infection. After primary infection, HCV RNA test results are depicted on the timeline by squares. Black squares represent HCV RNA–positive test results, and white squares represent HCV RNA–negative test results. Primary clearance is distinguished from viral suppression by the number of HCV RNA–negative test results (white squares). The top timelines (*i* and *ii*) depict primary clearance (as indicated by the 2 consecutive HCV RNA–negative test results), and the bottom timelines (*iii* and *iv*) depict viral suppression (defined as 1 HCV RNA–negative test result). In timelines *ii* and *iv*, HCV RNA was undetectable at the time of primary infection detection, as indicated by the white initial squares (HCV RNA tests) in the timelines. The estimated date of primary clearance is illustrated using a white triangle, and the estimated date of viral suppression is illustrated using a gray triangle. These dates are both estimated as follows: if HCV RNA is detectable at the time of detection of primary infection (*i* and *iii*), the estimated date of primary clearance or viral suppression is the midpoint between the HCV RNA–positive test result prior to primary clearance or viral suppression and the first HCV RNA–negative test result.

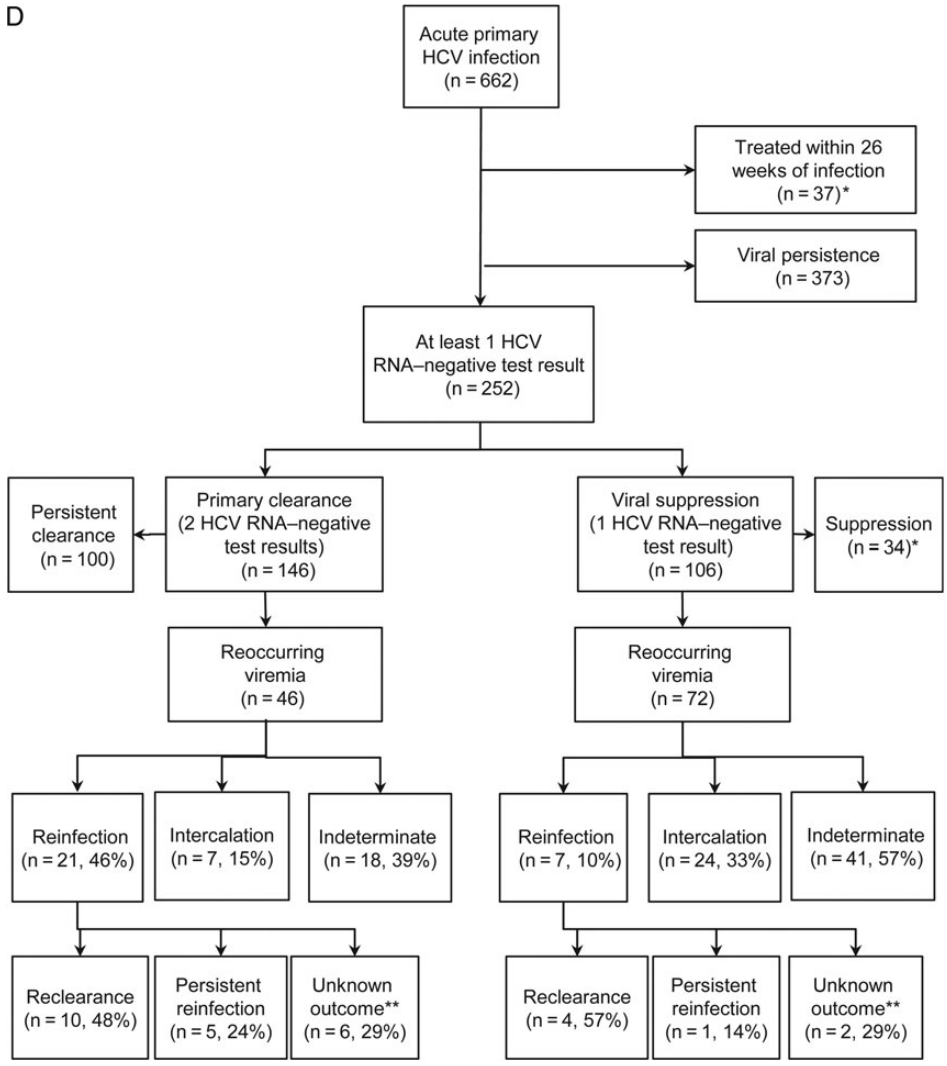

Figure 1 continued. If HCV RNA is undetectable at the time of detection of primary infection (*ii* and *iv*), the estimated date of primary clearance or viral suppression is the midpoint between the estimated date of primary infection (the beginning of the illustrated timeline) and the first HCV RNA–negative test result. In all 4 cases, the time to primary clearance or viral suppression is the time from the estimated date of infection until the estimated date of primary clearance or viral suppression. *C,* Estimated dates of and times to reinfection. Both timelines represent participants with confirmed primary HCV infection followed by either viral suppression or primary clearance and confirmed reinfection. The timelines begin at the estimated date of primary clearance or viral suppression. HCV RNA test results are depicted on the timeline by squares. Black squares represent HCV RNA–positive test results, and white squares represent HCV RNA–negative test results. Primary clearance is distinguished from viral suppression by the number of HCV RNA–negative test results (white squares). The top timeline (*i*) depicts primary clearance (as indicated by the 2 consecutive HCV RNA–negative test results), and the bottom timeline (*ii*) depicts viral suppression (define as 1 HCV RNA–negative test result). The estimated date of reinfection is the midpoint between the last HCV RNA–negative test result and the first HCV RNA–positive test result. The time to reinfection is defined as the time from the estimated date of primary clearance or viral suppression until the estimated date of reinfection. *D,* Flowchart of reinfection classification. *Primary HCV infection outcome unknown; **Reinfection outcome unknown: includes five cases with insufficient follow-up to determine outcome and three cases with change in genotype after reinfection.

was limited to studying HCV reinfection and reclearance in the absence of a history of antiviral treatment. Individuals who were treated >26 weeks after the estimated date of primary infection were censored from the treatment date. Individuals treated for HCV infection were excluded if the estimated duration of primary infection was <26 weeks, to reduce misclassification bias due to uncertainty around subsequent spontaneous clearance without treatment (n = 37).

Primary HCV Infection Outcomes

After acute primary HCV infection, subjects with 1 subsequent HCV RNA–negative test result and those with 2 consecutive HCV RNA tests (≥28 days apart) with negative results were classified as having viral suppression and primary clearance, respectively. Those with viral suppression at the final follow-up visit (n = 34) were excluded because the final outcome could not be determined. Those with detectable HCV RNA following

viral suppression or primary clearance were classified as having reoccurring viremia. Among those with reoccurring viremia, viral genotype/subtype and results of viral sequence analysis were used to distinguish reinfection (defined as detection of heterologous virus with no subsequent detection of the original viral strain), intercalation (defined as detection of homologous virus), and indeterminate cases (defined as cases for which viral sequencing data were unavailable or detection of heterologous virus with subsequent detection of the original viral strain). If primary clearance occurred prior to detection of seroconversion, such that no HCV RNA could be isolated from the primary infection, serotyping was performed to classify primary infection.

Estimated Dates and Times of Primary Clearance, Viral Suppression, and Reinfection

Methods for determining the estimated dates of primary clearance, viral suppression, and reinfection are illustrated in Figure 1*B*–*D*. In participants with evidence of primary clearance prior to reinfection, the time to reinfection was calculated as the time from the date of primary clearance to the date of reinfection. In participants with viral suppression prior to reinfection, the time to reinfection was calculated as the time from the date of viral suppression to the date of reinfection (Figure 1*C*). The time to reappearance of viremia in intercalation and indeterminate intermittent viremia was calculated similarly to the time to reinfection.

HCV Reinfection Outcomes

Reclearance was defined as 2 consecutive HCV RNA–negative tests (performed ≥28 days apart) with negative results following reinfection. The date of reclearance was calculated similarly to the date of primary clearance, and the time to reclearance was calculated as the time from the date of reinfection to the date of reclearance. For those without clearance, follow-up time was calculated from the date of reinfection until the date of the last therapy-naive positive HCV RNA test result. Persistent reinfection was defined as continuous viremia with the confirmed reinfecting virus for >6 months.

Classification of Peak HCV RNA Load During Primary Infection and Reinfection

Peak HCV RNA load was defined as the maximum quantitative RNA value measured within 3 months of the date of infection for both primary infection and reinfection (Supplementary Materials).

Statistical Analyses

Wilcoxon signed rank tests were used to evaluate the median within-participant difference between peak log HCV RNA load in reinfection, compared with primary infection. It was hypothesized that the peak log RNA load would be lower during reinfection, compared with primary infection [6]. Participants with at least 1 quantitative HCV RNA test in the first 3 months of primary HCV infection and reinfection were included.

Predictors of HCV reclearance were assessed using Cox proportional hazards regression. Models included shared frailty terms for study site to capture unobserved heterogeneity between sites that may have contributed to the time to reclearance. Potential interactions between study site—categorized as the Baltimore Before and After Acute Study of Hepatitis (BBAASH; the study contributing largest number of reinfection events) versus other sites—and hypothesized predictors were evaluated. Hypothesized predictors were determined a priori on the basis of established predictors of primary clearance, including age [21], sex [1, 15, 22, 23], *IFNL4* genotype (SNP rs12979860; CC vs CT/TT) [24–26], the combined effect of sex and *IFNL4* genotype (female rs12979860 CC vs others) [15, 23], HCV genotype during reinfection (genotype 1 vs non–genotype 1) [15, 27], and reinfection with the same versus a different HCV genotype from that in the primary infection. It was hypothesized that participants reinfected with the same HCV genotype would have a greater propensity toward reclearance [28, 29]. The combined effect of sex and *IFNL4* genotype was investigated by comparing females with the rs12979860 CC genotype and all other participants because, although this study combined the largest number of HCV reinfections studied to date, there were not sufficient numbers of reinfections to investigate the interaction between sex and *IFNL4* genotype. The effect of human immunodeficiency virus (HIV) infection [30] was not assessed owing to small numbers of HIV-infected participants. The effects of jaundice and elevated alanine aminotransferase levels were not assessed because most of the participating studies did not collect this information at the time of HCV reinfection.

Differences in times to primary clearance and reclearance in participants with reclearance were assessed using gap-time unrestricted proportional hazards regression, which is appropriate for analysis of predictors of time-to-event outcomes with multiple events [31]. The hypothesis was that time to primary clearance would be longer than the time to reclearance [6].

For all investigations, sensitivity analyses were performed to assess the effect of excluding participants infected with HIV at reinfection (n = 3), excluding reinfections defined on the basis of serotyping (n = 2), excluding reinfections with viral suppression rather than primary clearance prior to reinfection (n = 7), and stratifying by study site (BBAASH vs others). For the analysis of differences between peak HCV RNA loads in primary HCV infection and reinfection, sensitivity analysis of the effect of defining the peak RNA load as the peak within 1 month of infection, rather than 3 months, were conducted (Supplementary Materials). For the analysis of predictors of reinfection, sensitivity analyses excluding participants with <2 HCV RNA tests after reinfection were also performed.

Characteristics at the time of primary HCV infection and reappearance of viremia were analyzed by primary infection

Table 2. Characteristics, Exposures, and Risk Behaviors of 591 Participants With Acute Primary Hepatitis C Virus (HCV) Infection

Characteristic at Time of Incident Primary HCV Infection	Persistent HCV, Participants, No. (%) (n=373)	Cleared or Intermittent HCV Infection				
		Overall, Participants, No. (%) (n=218)	Reinfection	Intercalation	Indeterminate Intermittent Viremia	Persistent Cleared
Overall	. . .	. . .	28/218 (13)	31/218 (14)	59/218 (27)	100/218 (46)
Site						
ACS (the Netherlands)	18 (5)	24 (11)	4/24 (17)	1/24 (4)	10/24 (42)	9/24 (38)
ATAHC (Australia)	84 (23)	29 (13)	2/29 (7)	1/29 (3)	3/29 (10)	23/29 (79)
BAHSTION (United States)	21 (6)	21 (10)	1/21 (5)	3/21 (14)	6/21 (29)	11/21 (52)
BBAASH (United States)	52 (14)	59 (27)	15/59 (25)	20/59 (34)	12/59 (20)	12/59 (20)
HEPCO (Canada)	48 (13)	18 (8)	0/18 (0)	0/18 (0)	11/18 (61)	7/18 (39)
HITS-c (Australia)	6 (2)	3 (1)	0/3 (0)	0/3 (0)	0/3 (0)	3/3 (100)
HITS-p (Australia)	63 (17)	20 (9)	2/20 (10)	1/20 (5)	1/20 (5)	16/20 (80)
N2 (Australia)	10 (3)	7 (3)	2/7 (29)	3/7 (43)	0/7 (0)	2/7 (29)
UFO (United States)	71 (19)	37 (17)	2/37 (5)	2/37 (5)	16/37 (43)	17/37 (46)
Age, y, median (IQR)[a]	27 (23–34)	26 (22–30)	24 (20–30)	25 (24–28)	26 (21–30)	26 (23–32)
Sex						
Male	259 (69)[b]	118 (54)	14/118 (12)	16/118 (14)	38/118 (32)	50/118 (42)
Female	113 (30)	100 (46)	14/100 (14)	15/100 (15)	21/100 (21)	50/100 (50)
Data missing	1 (0)	0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)
Ethnicity/race						
European origin	305 (82)	177 (81)	26/177 (15)	25/177 (14)	48/177 (27)	78/177 (44)
Other	41 (11)	23 (11)	2/23 (9)	4/23 (17)	6/23 (26)	11/23 (48)
Data missing	27 (7)	18 (8)	0/18 (0)	2/18 (11)	5/18 (28)	11/18 (61)
History of IDU	358 (96)	210 (96)	28/210 (13)	30/210 (14)	59/210 (28)	93/210 (44)
HIV infection[a]						
No	328 (88)	199 (91)	25/199 (13)	28/199 (14)	55/199 (28)	91/199 (46)
Yes	30 (8)	12 (6)	3/12 (25)	1/12 (8)	2/12 (17)	6/12 (50)
Data missing	15 (4)	7 (3)	0/7 (0)	2/7 (29)	2/7 (29)	3/7 (43)
Peak HCV RNA load, log IU/mL, median (IQR)[c]	5.5 (4.5–6.4)	5.8 (4.0–7.0)	6.7 (5.3–7.0)	5.7 (5.1–6.8)	5.3 (2.9–6.5)	5.9 (3.2–7.0)
HCV genotype[a]						
1	174 (47)[d]	114 (52)	18/114 (16)	24/114 (21)	27/114 (24)	45/114 (39)
2	22 (6)	10 (5)	1/10 (10)	2/10 (20)	2/10 (20)	5/10 (50)
3	130 (35)	46 (21)	8/46 (17)	5/46 (11)	9/46 (20)	24/46 (52)
4	2 (1)	4 (2)	1/4 (25)	0/4 (0)	3/4 (75)	0/4 (0)
6	4 (1)	0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)
Mixed	10 (3)	2 (1)	0/2 (0)	0/2 (0)	0/2 (0)	2/2 (100)
Unknown	31 (8)	42 (19)	0/42 (0)	0/42 (0)	18/42 (43)	24/42 (57)
Recent IDU[e]						
No	57 (15)	19 (9)	2/19 (11)	2/19 (11)	1/19 (5)[f]	14/19 (74)
Yes	238 (64)	113 (52)	9/113 (8)	5/113 (4)	40/113 (35)	59/113 (52)
Data missing	5 (1)	6 (3)	1/6 (17)	1/6 (17)	0/6 (0)	4/6 (67)
Data not collected at cohort site	73 (20)	80 (37)	16/80 (20)	23/80 (29)	18/80 (23)	23/80 (29)
Recent IDU frequency[e]						
No recent injecting	41 (11)	16 (7)	1/16 (6)	2/16 (13)	1/16 (6)	12/16 (75)
Daily or more	127 (34)	48 (22)	7/48 (15)	3/48 (6)	13/48 (27)	25/48 (52)
Less than daily but at least weekly	72 (19)	48 (22)	3/48 (6)	1/48 (2)	19/48 (40)	25/48 (52)

Table 2 continued.

Characteristic at Time of Incident Primary HCV Infection	Persistent HCV, Participants, No. (%) (n=373)	Cleared or Intermittent HCV Infection				
		Overall, Participants, No. (%) (n=218)	Reinfection	Intercalation	Indeterminate Intermittent Viremia	Persistent Cleared
Less than weekly	55 (15)	19 (9)	0/19 (0)	0/19 (0)	8/19 (42)	11/19 (58)
Data missing	5 (1)	7 (3)	1/7 (14)	2/7 (29)	0/7 (0)	4/7 (57)
Data not collected at cohort site	73 (20)	80 (37)	16/80 (20)	23/80 (29)	18/80 (23)	23/80 (29)
Primary drug injected recently[e,g]						
Heroin/other opioids	122 (33)	72 (33)	5/72 (7)	3/72 (4)	26/72 (36)	38/72 (53)
Psychostimulants	88 (24)	34 (16)	3/34 (9)	1/34 (3)	14/34 (41)	16/34 (47)
Other	6 (2)	0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)
Data missing	84 (23)	32 (15)	4/32 (13)	4/32 (13)	1/32 (3)	23/32 (72)
Data not collected at cohort site	73 (20)	80 (37)	16/80 (20)	23/80 (29)	18/80 (23)	23/80 (29)
Recent receptive needle sharing[e]						
No	162 (43)	77 (35)	7/77 (9)	1/77 (1)	26/77 (34)	43/77 (56)
Yes	75 (20)	30 (14)	3/30 (10)	3/30 (10)	11/30 (37)	13/30 (43)
Data missing	63 (17)	31 (14)	2/31 (6)	4/31 (13)	4/31 (13)	21/31 (68)
Data not collected at cohort site	73 (20)	80 (37)	16/80 (20)	23/80 (29)	18/80 (23)	23/80 (29)

Data are proportion (%) of participants, unless otherwise indicated. Data exclude 34 participants who did not have intermittent viremia or spontaneous clearance but had a negative result of their final HCV RNA test (ie, viral suppression) and 37 participants treated within 26 weeks of infection.

Abbreviations: ACS, Amsterdam Cohort Studies; ATAHC, Australian Trial in Acute Hepatitis C; BAHSTION, Boston Acute HCV Study: Transmission, Immunity, and Outcomes Network; BBAASH, Baltimore Before and After Acute Study of Hepatitis; HCV, hepatitis C virus; HEPCO, St. Luc Cohort; HITS-c, Hepatitis C Incidence and Transmission Study–Community; HITS-p, Hepatitis C Incidence and Transmission Study–Prison; HIV, human immunodeficiency virus; IDU, injection drug use; IQR, interquartile range; N2, Networks 2; UFO, UFO study.

[a] At the time of primary HCV infection.

[b] Statistically significant differences from the group with persistent spontaneous clearance, the group with reinfection, and the group with intercalation.

[c] In the first 3 months of primary HCV infection.

[d] Statistically significant difference in the genotype distribution (genotype 1 vs other genotypes) from the group with intercalation.

[e] Reported at the interview prior to primary HCV infection diagnosis, recent indicates last 1–6 months prior to interview.

[f] Statistically significant differences from the group with persistent infection and the group persistent spontaneous clearance.

[g] Heroin/other opioids includes heroin, other opioids, and speedball; psychostimulants include amphetamines (including methamphetamines) and cocaine.

outcome, using Kruskal–Wallis, χ^2, and Fisher exact tests, as appropriate. Proportions of reinfections resulting in spontaneous clearance and persistent infection 6 months after reinfection were estimated using Kaplan–Meier survivor functions. Finally, classifications of reoccurring viremia after primary clearance versus reoccurring viremia after viral suppression were compared using χ^2 tests. Statistically significant differences were those with P values of <.05; P values are 2-sided. All analyses were performed using Stata, version 11.0 (College Station, Texas). Participant timeline figures were prepared in R [32, 33].

RESULTS

Participant Characteristics

Of 662 participants with acute primary HCV infection, 591 had a defined infection outcome (Figure 1*D* and Table 2). At the time of primary infection, the median age was 26 years, and 36% of participants were female. Most participants (96%) had injected drugs. A small minority (7%) were infected with HIV.

Primary HCV Infection Characteristics and Outcomes

Among those with known HCV genotypes during primary infection (518 [88%]), the most common genotypes were 1 (in 288 participants [56%]) and 3 (in 176 [34%]; Table 2). Following primary infection, 252 participants had viral suppression (at least 1 negative HCV RNA test result), of whom 146 (58%) had primary clearance (at least 2 consecutive HCV RNA tests performed ≥28 days apart that had negative results). Overall, 118 (47%) had reoccurring viremia, 72 after viral suppression and 46 after primary clearance (Figure 1*D*). Among those with reoccurring viremia, viral sequence analysis was used to distinguish reinfection (in 28 participants), intercalation (in 31), and indeterminate cases (in 55, viral sequencing data were not available; and in 4, heterologous virus with subsequent detection of the original viral strain was observed). Reinfection was more common

Table 3. Viral Genotype Detected During Primary Hepatitis C Virus (HCV) Infection and Reinfection Among 28 Participants With Reinfection

Characteristic at Time of HCV Infection	Primary HCV Infection (n = 28)	HCV Reinfection (n = 28)
HCV genotype		
1	18 (64)	17 (61)
2	1 (4)	4 (14)
3	8 (29)	6 (21)
4	1 (4)	1 (4)
HCV genotype/subtype in primary infection vs reinfection		
Different genotype	. . .	15 (54)
Different subtype	. . .	3 (11)
Same genotype	. . .	10 (36)

after primary clearance (46% of cases) than viral suppression (10% of cases; $P < .001$; Figure 1*D*).

Study Retention and Frequency of HCV Testing

In the 28 reinfected participants, the median length of follow-up after the date of primary HCV infection was 4.6 years (interquartile range [IQR], 3.2–7.3 years) years. The median number of HCV RNA tests in this time was 17 (IQR, 9–35), and the median interval between tests was 48 days (IQR, 33–122 days). The median interval between tests prior to primary clearance (49 days; IQR, 32–140 days) was similar to the median interval during the risk period for reinfection and until reclearance or the end of follow-up (53 days; IQR, 33–138 days). In participants without reinfection, the median length of follow-up was 1.5 years (IQR, 0.7–2.8 years), the median number of HCV RNA tests was 5 (IQR, 3–9), and the median interval between tests was 84 days (IQR, 38–140 days). HCV RNA assay lower limits are provided in Supplementary Table 3.

HCV Reinfection

Twenty-eight participants had at least 1 reinfection (Figure 1*D*), and the incidence rate was 12.3 cases/100 person-years (95% CI, 8.5–17.8 cases/100 person-years); data were calculated by including participants with persistent primary clearance and reinfection, and Table 1 shows incidence rates stratified by study site. Fifteen reinfections involved a viral genotype different from that detected during primary infection, 3 involved a different viral subtype, and 10 involved the same genotype and subtype (Table 3).

Peak HCV RNA Load in Reinfection, Compared With Primary Infection

Fifteen reinfections had quantitative HCV RNA measurements available within the first 3 months of both primary infection and reinfection. The median peak HCV RNA load in reinfection (3.4 log IU/mL; IQR, 2.6–6.5 log IU/mL) was lower than that in primary infection (6.7 log IU/mL [IQR, 5.3–7.0 log IU/mL]; median difference, 1.46 log IU/mL [IQR, 0.34–4.18 log IU/mL]; $P = .011$). Timelines for quantitative HCV RNA measurements for 3 participants with reinfection, illustrating a range of trajectories, are included in Supplementary Figure 1.

Reinfection Outcomes

For 23 of the 28 reinfection cases, follow-up was sufficient (defined as the occurrence of at least 2 subsequent study visits) to classify the outcome. Of the 9 participants with reinfection without reclearance and with sufficient follow-up, the median estimated duration of reinfection at the end of follow-up was 66.9 months (range, 10.1–226.6 months). All participants had HCV genotype data from >1 time point following reinfection. The Kaplan–Meier estimate of the reclearance proportion 6 months after reinfection was 52% (95% CI, 33%–73%), after excluding the 3 individuals with changes in genotype or subtype.

Time to Reclearance

The median time to reclearance after reinfection was 3.0 months (IQR, 2.0–4.4 months). In the same participants, the median time to primary clearance was 5.5 months (IQR, 2.6–11.2 months).There was a tendency toward a shorter time to reclearance, compared with the time to primary clearance; this did not reach statistical significance (hazard ratio [HR], 1.86; 95% CI, .70–4.91; $P = .211$).

Predictors of HCV Reclearance

In shared frailty (for cohort site) but otherwise unadjusted Cox proportional hazards analysis of participants with reinfection, female participants with *IFNL4* rs12979860 CC genotype were 4-fold more likely to reclear at any given time, compared with other participants (HR, 4.16; 95% CI, 1.24–13.94; $P = .021$; Table 4). There were no other statistically significant factors associated with reclearance.

Multiple HCV Reinfections

Of the 14 participants with reclearance, 5 had further reinfections. Three participants had 2 reinfections, and 2 participants had 3 reinfections. Overall, 35 reinfections were observed (Figure 2).

HCV Intercalation

Compared with reinfection, intercalation was more likely to occur after a shorter period of HCV RNA undetectability ($P < .001$; Table 5). Similarly, intercalation tended to be observed earlier following primary infection (timing of reappearance of viremia after the estimated date of primary HCV infection in intercalation, $P = .002$; Table 5). However, considerable variation was observed.

Indeterminate Intermittent Viremia

Of the 59 cases of indeterminate intermittent viremia, 4 were classified on the basis of sequencing of heterologous virus

Table 4. Cox Proportional Hazards Regression Analysis of Predictors of Reclearance of Hepatitis C Virus (HCV), Adjusted for Cohort Site by Using a Shared Frailty Model

Predictor	Reclearances, No.	Reclearance Rate, Cases/100 Person-Years	HR (95% CI)	*P* Value	Shared Frailty for Cohort	
					θ	*P* Value
Overall	14	56.6	. . .		. . .	
Age at reinfection, y						
≤25	7	45.1	1.00		. . .	
>25	5	60.9	1.72 (.45–6.53)	.426	0.72	.119
Sex[a]						
Male	5	36.0	1.00		. . .	
Female	9	82.9	2.45 (.72–8.29)	.150	0.74	.125
IFNL4 rs12979860						
CC	10	69.8	2.00 (.56–7.18)	.285	1.18	.053
CT/TT	4	38.3	1.00		. . .	
Combined effect of sex and *IFNL4*[b]						
Female and CC	7	126.9	4.16 (1.24–13.94)	.021	1.08	.063
Other	7	36.4	1.00		. . .	
Reinfection HCV genotype						
1	9	53.2	1.00		. . .	
Other	5	63.9	2.79 (.62–12.51)	.181	1.26	.041
Reinfected with different genotype from that in primary infection						
Yes	6	53.1	4.69 (.90–24.36)	.066	2.22	.017
No	8	59.5	1.00			

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] Data are missing for 2 participants with reclearance. No data were missing data for participants without reclearance.

[b] Schoenfeld residuals were used to evaluate the proportional hazards assumption. $P = .990$ by the test of proportional hazards.

with subsequent detection of the original viral strain, and the remaining 55 were classified on the basis of insufficient viral sequencing. The duration of the period of HCV RNA undetectability preceding reappearance of viremia and the timing of reappearance of viremia in the latter 55 cases were similar to that in intercalation (Table 5).

Sensitivity Analyses

Results were not sensitive to any of the factors tested (Supplementary Tables 3–5).

DISCUSSION

This study characterizes the natural history of viral suppression, primary clearance, HCV reinfection and reclearance in the largest sample of participants (mostly people who inject drugs) with well-defined primary HCV infection and reinfection reported to date. The peak HCV RNA load at the time of HCV reinfection was lower than in primary infection, providing further evidence of protective immunity in humans. Six months after reinfection, the clearance proportion was 52% (95% CI, 33%–73%). The combined effect of female sex and rs12979860 CC *IFNL4* genotype was predictive of reclearance following reinfection.

Reclearance was predicted by a combined effect of sex and *IFNL4* genotype. To the best of our knowledge, this is the first study to investigate predictors of reclearance. The propensity toward reclearance was 4 times greater among females with the rs12979860 CC *IFNL4* genotype. This is particularly notable because, by definition, all participants with reinfection have already cleared 1 HCV infection and therefore would be expected to have a greater tendency toward spontaneous clearance a priori. In the context of primary clearance, similar findings have been reported with respect to female sex [1, 22] and *IFNL4* genotype [24, 25] predicting clearance independently and in combination [15, 23], including within the InC^3 study population [15]. The *IFNL4* gene region encodes the interferon λ3 protein and is involved in viral control, although the precise mechanism remains unknown. It is possible that female sex influences HCV clearance through a mechanism related to general sex-based differences in immunity [34, 35]; however, the pathways by which these differences affect HCV control require elucidation. Further research is required to assess whether the combined effect of *IFNL4* genotype and sex on primary clearance and reclearance is simply the product of the 2 independent effects or whether there is a synergistic effect [15, 23]. The importance of sex and *IFNL4* genotype in both primary HCV

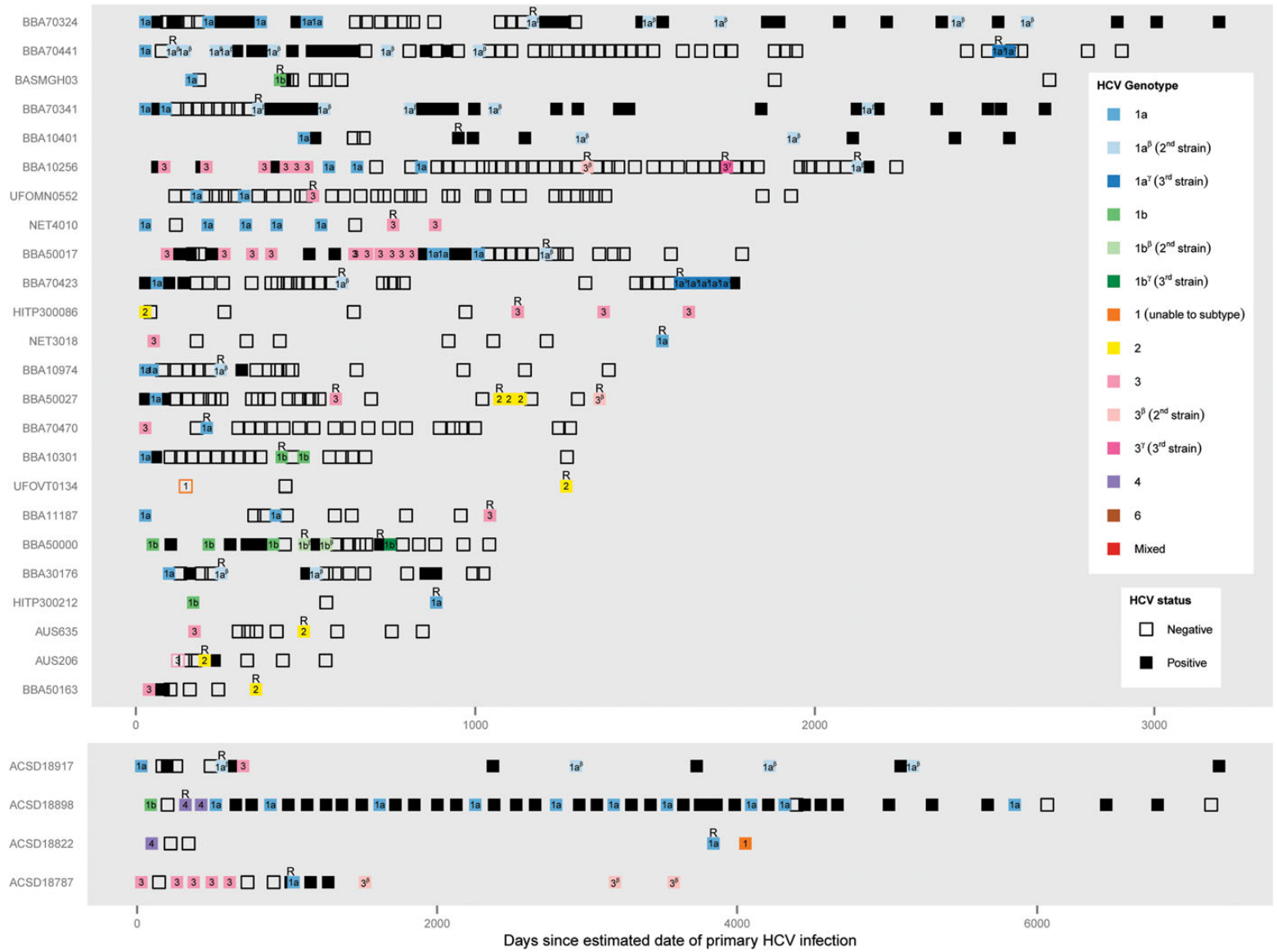

Figure 2. Timeline of reinfection events from the estimated date of primary hepatitis C virus (HCV) infection. Timelines illustrate results of HCV RNA tests and HCV genotyping for the 28 participants with reinfection. Each box represents 1 HCV RNA test. Empty boxes are tests in which HCV RNA was undetectable, whereas filled boxes (black or colored) indicate that HCV RNA was detectable. HCV genotyping results are indicated using color and box labels. Within individuals, distinct strains within a single viral genotype and subtype that were confirmed by viral sequencing are illustrated using different shades of the same color. Reinfection events (R) are defined by the appearance of a new viral genotype, viral subtype, or distinct strain confirmed by viral sequencing following a period during which HCV RNA was undetectable. HCV from the primary infections of participants UFOVT0134 and AUS206 was serotyped.

Table 5. Time From Estimated Date of Primary Infection Until Reappearance of Viremia, Number of Hepatitis C Virus (HCV) RNA–Negative Test Results, and Duration of Preceding Period of HCV RNA Undetectability

	No.	Median	IQR	Range
Time to reappearance of viremia, d[a]				
Intercalation	31	250	174–433	113–2219
Reinfection	28	546	312–984	93–3747
Indeterminate cases without sufficient viral sequencing	55	231	140–399	46–3167
Indeterminate cases with viral sequencing[b]	4	173	168–645	166–1114
HCV RNA–negative test results, no.[c]				
Intercalation	31	1	1–2	1–6
Reinfection	28	3	2–7	1–15
Indeterminate cases without sufficient viral sequencing	55	1	1–2	1–18
Indeterminate cases with viral sequencing[b]	4	1	1–6	1–10
Duration of HCV undetectability, d[d]				
Intercalation	7	88	32–221	31–1293
Reinfection	21	210	131–412	28–1031
Indeterminate cases without sufficient viral sequencing	22	119	63–282	28–2707
Indeterminate cases with viral sequencing[b]	1	603	. . .	. . .

Abbreviation: IQR, interquartile range.

[a] Statistically significant differences between the reinfection group and the intercalation group ($P = .002$) and between the reinfection group and indeterminate group without sufficient viral sequencing ($P < .001$).

[b] Classified as indeterminate on the basis of detection of heterologous virus with subsequent detection of the original viral strain.

[c] Preceding reappearance of viremia; statistically significant differences between the reinfection group and the intercalation group ($P = .001$) and between the reinfection group and indeterminate group without sufficient viral sequencing ($P < .001$).

[d] Among cases with at least 2 HCV RNA–negative test results at least 28 days apart, calculated as the period from the first negative test result until the last negative test result.

infection and reinfection suggests that these factors have a crucial role in long-term protection from persistent HCV infection. Although *IFNL4* genotype and sex are fixed genetic traits, the fact that spontaneously clearing infections are controlled better with subsequent exposures suggests the existence of an adaptive component. A better understanding of the mechanisms behind the immune response in females with the *IFNL4* rs12979860 CC genotype has the potential to provide insights for vaccine development.

The findings presented here suggest partial protective immunity following primary clearance of HCV. The peak HCV RNA level was lower during reinfection, compared with that during primary infection. Reclearance following reinfection was observed in half of the participants, with the time to reclearance tending to be shorter following reinfection, compared with that for primary clearance. These findings are consistent with previous findings by Osburn et al and are not sensitive to stratification or adjustment by study site (BBAASH vs others) [6]. Mathematical modeling studies have shown that the interval between tests influences the proportions of reinfections resulting in reclearance and persistence [13]. In this study, heterogeneity in test intervals between study sites limited the interpretation of these proportions. Nonetheless, the identification of persistent reinfections indicates that, while primary HCV infection appears to confer protection against persistent HCV reinfection in some cases, there are limits to this protection. Chimpanzee studies indicate inadequate cross-strain protection in some cases [28]; however, this study did not find a higher probability of reclearance in participants reinfected with the same genotype as that found during their primary infection. Further studies to characterize the viral genomes of the primary and reinfection strains and to resolve the detailed characteristics of the immune responses against these viruses, including both neutralizing antibodies and HCV-specific T cells, are warranted.

The identification of diverse outcomes of HCV reinfection illustrates the complexity of the natural history of HCV. Among the 23 participants with follow-up after HCV reinfection, approximately one third experienced persistent reinfection, and one third experienced multiple reinfections after resolution of their first reinfection. The remaining third was composed of participants with reclearance but without further reinfections (possibly partly due to shorter follow-up) and of participants with changes in viral genotype or subtype following reinfection. This report adds to the few cases of multiple consecutive reinfection that have been reported previously [5, 6, 11], highlighting the ongoing risk of reinfection among those who continue to be exposed to HCV and emphasizing the need for education about reinfection risk in these groups and for delivery of HCV antiviral treatment to those who become reinfected.

In contrast to reinfection, intercalation was usually observed within the first 2 years of primary HCV infection, consistent with previous reports of fluctuations in HCV RNA loads in early HCV infection [36, 37]. However, intercalation cases were also observed later in HCV infection and after lengthier periods of HCV RNA undetectability, as has previously been reported [6, 12, 38, 39]. This highlights the importance of viral sequencing for classification of HCV reinfection. Intercalation may occur as a result of transient control of viral replication by the host immune response, but further research is required to develop a more detailed understanding of such events.

Despite bringing together the largest number of well-defined HCV reinfection events following spontaneous clearance or viral suppression reported to date, the number of events is low for detailed statistical analysis. Therefore, our analysis of predictors of HCV reclearance could not be adjusted for the effect of potential confounders. Participants with identified

reinfections were typically followed longer and more frequently than other participants, and a large proportion of reoccurring viremia events could not be classified as reinfections or intercalations. These factors suggest that the true reinfection rate in the InC^3 population is likely to be higher than the observed rate. While standard methods were used to classify outcomes of infection, there were differences between cohort sites in terms of methods of recruitment, test intervals, HCV RNA monitoring methods, and the region of HCV sequenced to assess reinfection. In some of the participating cohorts, data on HCV-related risk behaviors were not collected; therefore, risk behaviors could not be assessed as predictors of HCV reinfection or reclearance. The analysis of time to HCV reclearance versus primary clearance only included participants with reclearance during the study period. While the participants with persistent reinfection were all followed for at least 10 months, indicating that future reclearance would be unlikely, late clearance can occur, so there is a small risk of bias from excluding right-censored data (Supplementary Materials) [39].

This is the first study to investigate predictors of HCV reclearance. Similar to primary clearance, there appears to be a combined effect of sex and *IFNL4* genotype on reclearance of HCV, suggesting that these factors together have considerable impact on long-term protection from persistent HCV infection. This study also highlights the complexity of acute HCV infection and reinfection and the factors that contribute to viral clearance. These findings suggest that HCV reinfection is associated with lower levels of viremia and a possibly shorter time to spontaneous reclearance, supporting a role for immunologic memory in conferring partial protection against persistent infection. Nonetheless, there is considerable heterogeneity in reinfection outcomes, and participants with ongoing exposure to HCV risk developing persistent reinfection.

STUDY GROUP MEMBERS

Steering committee: Kimberly Page (chair, UFO), Julie Bruneau (HEPCO), Andrea L. Cox (BBAASH), Gregory J. Dore (ATAHC), Jason Grebely (ATAHC), Margaret Hellard (N2), Georg Lauer (BAHSTION), Arthur Y. Kim (BAHSTION), Andrew R. Lloyd (HITS-p), Lisa Maher (HITS-c), Barbara H. McGovern (BAHSTION), Maria Prins (ACS), and Naglaa H. Shoukry (HEPCO). Coordinating center: Meghan Morris (study coordinator), Judy Hahn (coinvestigator), and Thomas M. Rice (data manager). Site data managers: Maryam Alavi (ATAHC), Rachel Bouchard (HEPCO), Jennifer Evans (UFO), Bart Grady (ACS), Jasneet Aneja (BAHSTION), Rachel Sacks-Davis (Networks 2), Suzy Teutsch (HITS-p), Bethany White (HITS-c), Brittany Wells (BBAASH), and Geng Zang (HEPCO). InC^3 researchers: Tanya Applegate, Gail Matthews, and Barbara Yeung (ATAHC); Bart Grady and Thijs van de Laar (ACS); Jasneet Aneja and Leslie Erin Prince (BAHSTION); Elise Roy and Geng Zang (HEPCO); Anna Bates, Jarliene Enriquez, Sammy Chow, and Ju Park (HITS-c); Luke McCredie and Suzy Teutsch (HITS-p); Campbell Aitken, Scott Bowden, Peter Higgs, and Lilly Tracy (N2); and Alya Briceno (UFO).

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Drug Abuse or the National Institutes of Health.

Financial support. This work was supported by the National Institutes of Health (NIH; grants U19 AI088791 [to A. L. C.] and U19 AI066345 [to A. Y. K., T. M. A. and B. H. M.]); the National Institute on Drug Abuse, NIH (grants R01 DA031056 [to the InC^3 Study], R01 DA033541 [to A. Y. K.], R01 DA016017 [to K. P. and M. M.], and R01 DA15999-01 [to G. J. D., J. G., A. R. L., and M. H.]); the Victorian Operational Infrastructure Support Program (to the Burnet Institute); the National Health and Medical Research Council (postgraduate scholarship to R. S.-D., practitioner research fellowships to G. J. D. and A. R. L., senior research fellowships to M. H. and L. M., career development fellowship to J. G., and project grants 630483 [to the Hepatitis C Virus Incidence and Transmission Study–Community {HITS-c}] and 331312 [to the Networks 2 {N2} project]); Fonds de la Recherche du Québec–Santé (research career awards to J. B. and N. H. S.); an Australian postgraduate PhD award (to B. H.); the Canadian Institutes of Health Research (grants MOP-103138 and MOP-106468 to J. B. and N. H. S.); the Netherlands National Institute for Public Health and the Environment (to the Amsterdam Cohort Study); the University of New South Wales Hepatitis C Vaccine Initiative (to the HITS-c); the Australian Centre for HIV and Hepatitis Virology Research (to the N2 project); and the Centre for Research Excellence into Injecting Drug Use (to M. H., G. J. D., L. M., J. G., and R. S. D.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat **2006**; 13:34–41.
2. Grebely J, Prins M, Hellard M, et al. Towards a hepatitis C virus vaccine: insights from studies of hepatitis C virus reinfection in injection drug users. Lancet Infect Dis **2012**; 12:408–14.
3. Aitken CK, Lewis J, Tracy SL, et al. High incidence of hepatitis C virus reinfection in a cohort of injecting drug users. Hepatology **2008**; 48:1746–52.
4. Micallef JM, Macdonald V, Jauncey M, et al. High incidence of hepatitis C virus reinfection within a cohort of injecting drug users. J Viral Hepat **2007**; 14:413–8.
5. van de Laar TJW, Molenkamp R, van den Berg C, et al. Frequent HCV reinfection and superinfection in a cohort of injecting drug users in Amsterdam. J Hepatol **2009**; 51:667–74.
6. Osburn WO, Fisher BE, Dowd KA, et al. Spontaneous control of primary hepatitis C virus infection and immunity against persistent reinfection. Gastroenterology **2010**; 138:315–24.

7. Pham ST, Bull RA, Bennett JM, et al. Frequent multiple hepatitis C virus infections among injection drug users in a prison setting. Hepatology **2010**; 52:1564–72.
8. Grebely J, Conway B, Raffa JD, Lai C, Krajden M, Tyndall MW. Hepatitis C virus reinfection in injection drug users. Hepatology **2006**; 44:1139–45.
9. Currie SL, Ryan JC, Tracy D, et al. A prospective study to examine persistent HCV reinfection in injection drug users who have previously cleared the virus. [erratum appears in Drug Alcohol Depend. 2008 Jul;96(1–2):192]. Drug Alcohol Depend **2008**; 93:148–54.
10. Mehta SH, Cox A, Hoover DR, et al. Protection against persistence of hepatitis C. Lancet **2002**; 359:1478–83.
11. Sacks-Davis R, Aitken C, Higgs P, et al. High Rates of Hepatitis C Virus Reinfection and Spontaneous Clearance of Reinfection in People who Inject Drugs: a Prospective Cohort Study. PLoS One **2013**; 8:e80216.
12. Page K, Osburn W, Evans J, et al. Frequent Longitudinal Sampling of Hepatitis C Virus Infection in Injection Drug Users Reveals Intermittently Detectable Viremia and Reinfection. Clin Infect Dis **2013**; 56:405–13.
13. Vickerman P, Grebely J, Dore GJ, et al. The more you look the more you find - Effects of hepatitis C virus testing interval on re-infection incidence and clearance: Implications for future vaccine study design. J Infect Dis **2012**; 205:1342–50.
14. Grebely J, Morris MD, Rice TM, et al. Cohort Profile: The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts (InC3) Study. Int J Epidemiol **2012**; 42:1649–59.
15. Grebely J, Page K, Sacks-Davis R, et al. The effects of female sex, viral genotype, and IL28B genotype on spontaneous clearance of acute hepatitis C virus infection. Hepatology **2014**; 59:109–20.
16. Dev AT, McCaw R, Sundararajan V, Bowden S, Sievert W. Southeast Asian patients with chronic hepatitis C: the impact of novel genotypes and race on treatment outcome. Hepatology **2002**; 36:1259–65.
17. Ray S, Arthur R, Carella A, Bukh J, Thomas DL. Genetic epidemiology of hepatitis C virus throughout Egypt. J Infect Dis **2000**; 182:698–707.
18. Van de Laar T, Langendam M, Bruisten S, et al. Changes in risk behavior and dynamics of hepatitis C virus infections among young drug users in Amsterdam, the Netherlands. J Med Virol **2005**; 77:509–18.
19. Tu E, Bull R, Greening G, et al. Epidemics of gastroenteritis during 2006 were associated with the spread of norovirus GII.4 variants 2006a and 2006b. Clin Infect Dis **2008**; 46:413–20.
20. Kuntzen T, Timm J, Berical A, et al. Viral Sequence Evolution in Acute Hepatitis C Virus Infection. J Virol **2007**; 81:11658–68.
21. Zhang M, Rosenberg PS, Brown DL, et al. Correlates of spontaneous clearance of hepatitis C virus among people with hemophilia. Blood **2006**; 107:892–7.
22. Page K, Hahn JA, Evans J, et al. Acute hepatitis C virus infection in young adult injection drug users: a prospective study of incident infection, resolution, and reinfection. J Infect Dis **2009**; 200:1216–26.
23. van den Berg CHBS, Grady BPX, Schinkel J, et al. Female Sex and IL28B, a Synergism for Spontaneous Viral Clearance in Hepatitis C Virus (HCV) Seroconverters from a Community-Based Cohort. PLoS One **2011**; 6:e27555.
24. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature **2009**; 461:798–801.
25. Tillmann HL, Thompson AJ, Patel K, et al. A polymorphism near IL28B is associated with spontaneous clearance of acute hepatitis C virus and jaundice. Gastroenterology **2010**; 139:1586–92, 92 e1.
26. Grebely J, Petoumenos K, Hellard M, et al. Potential role for IL28B genotype in treatment decision-making in recent hepatitis C virus infection. Hepatology **2010**; 52:1216–24.
27. Thomson EC, Fleming VM, Main J, et al. Predicting spontaneous clearance of acute hepatitis C virus in a large cohort of HIV-1-infected men. Gut **2011**; 60:837–45.
28. Prince AM, Brotman B, Lee D-H, et al. Protection against Chronic Hepatitis C Virus Infection after Rechallenge with Homologous, but Not Heterologous, Genotypes in a Chimpanzee Model. J Infect Dis **2005**; 192:1701–9.
29. Lanford R, Guerra B, Chavez D, et al. Cross-genotype immunity to hepatitis C virus. J Virol **2004**; 78:1575–81.
30. Thomas DL, Astemborski J, Rai RM, et al. The natural history of hepatitis C virus infection: host, viral, and environmental factors. JAMA **2000**; 284:450–6.
31. Kelly PJ, Lim LL-Y. Survival analysis for recurrent event data: an application to childhood infectious diseases. Stat Med **2000**; 19:13–33.
32. Wickham H. ggplot2: elegant graphics for data analysis. New York: Springer, **2009**.
33. R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, **2011**.
34. Bouman A, Heineman M, Faas M. Sex hormones and the immune response in humans. Hum Reprod Update **2005**; 11:411–23.
35. Klein S, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. Lancet Infectious Diseases **2010**; 10:338–49.
36. Hajarizadeh B, Grebely J, Applegate T, et al. Dynamics of HCV RNA levels during acute hepatitis C virus infection. J Med Virol **2014**; 86:1722–9.
37. Glynn SA, Wright DJ, Kleinman SH, et al. Dynamics of viremia in early hepatitis C virus infection. Transfusion **2005**; 45:994–1002.
38. Cox AL, Netski DM, Mosbruger T, et al. Prospective evaluation of community-acquired acute-phase hepatitis C virus infection. Clin Infect Dis **2005**; 40:951–8.
39. Mosley JW, Operskalski EA, Tobler LH, et al. The course of hepatitis C viraemia in transfusion recipients prior to availability of antiviral therapy. J Viral Hepat **2008**; 15:120–8.
40. Page-Shafer K, Pappalardo BL, Tobler LH, et al. Testing strategy to identify cases of acute hepatitis C virus (HCV) infection and to project HCV incidence rates. J Clin Microbiol **2008**; 46:499–506.
41. Hofer H, Watkins-Riedel T, Janata O, et al. Spontaneous viral clearance in patients with acute hepatitis C can be predicted by repeated measurements of serum viral load. Hepatology **2003**; 37:60–4.