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Induction of CXCR3- and CCR5-associated Chemokines during Acute Hepatitis C Virus Infection

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Abstract

Background and Aims—Characterization of inflammatory mediators, such as chemokines, during acute hepatitis C virus (HCV) infection might shed some light on viral clearance mechanisms.

Methods—Plasma levels of CXCR3 (CXCL9-11)- and CCR5 (CCL3-4)-associated chemokines, ALT and HCV RNA were measured in nine injection drug users (median 26 samples/patient) before and during ten acute (eight primary and two secondary) HCV infections. Using functional data analysis, we estimated smooth long-term trends in chemokine expression levels to obtain the magnitude and timing of over-all changes. Residuals were analyzed to characterize short-term fluctuations.

Results—CXCL9-11 induction began 38–53 days and peaked 72–83 days after virus acquisition. Increases in ALT levels followed a similar pattern. Substantial negative autocorrelations of chemokine levels at one week lags suggested substantial week-to-week oscillations. Significant correlations were observed between CXCL10 and HCV RNA as well as ALT and CXCR3-associated chemokines measured in the preceding week, CCL3-4 expression levels did not change appreciably during acute HCV infection.

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Conclusions—Elevation of CXCR3-associated chemokines late during acute HCV infection suggests a role for cellular immune responses in chemokine induction. Week-to-week oscillations of HCV RNA, chemokines, and ALT suggest frequent, repeated cycles of gain and loss of immune control during acute hepatitis C.

Keywords

CXCL9; CXCL10; CXCL11; CCL3; CCL4; inflammation

Introduction

Hepatitis C virus (HCV) infection affects an estimated 170 million individuals worldwide and ~5 million individuals in the United States [1–3]. Among individuals who are exposed to HCV, the majority (50–80%) will develop chronic infection that may result in cirrhosis and/or hepatocellular carcinoma. In order to establish a chronic infection, the virus must successfully evade host immune responses. The mechanisms leading to viral eradication are presently not understood although data support a role for both innate and adaptive immune responses as being required for viral clearance [4]. High levels of interferon α/β stimulated genes have been detected in the liver of acutely infected chimpanzees [5,6] although expression levels do not differentiate animals who clear the virus from those in whom it persists [6–8]. In contrast, disruption of innate immune pathways by structural (core, E2) and non-structural (NS3, NS5A) HCV proteins supports a role for innate immune responses in viral clearance [9]. With regard to the role of the adaptive immune response, vigorous and multi-specific CD4⁺ and CD8⁺ T-cell responses that appear in acute infection and persist overtime have been associated with viral resolution [10,11]. In addition, depletion studies in chimpanzees confirmed the crucial role of these cells in viral clearance [12,13].

Chemokines are small chemotactic cytokines that attract cells to inflammatory sites. Thus far, CXCR3-associated chemokines, CXCL9-11, and CCR5-associated chemokines, CCL3-5, appear to be particularly important in chronic HCV infection by promoting the development of intrahepatic inflammation leading to fibrogenesis [14]. We have recently shown that intrahepatic and peripheral levels of CXCR3-associated chemokines are significantly elevated in patients with advanced necroinflammatory activity and fibrosis [15,16]. Of the three chemokines, intrahepatic mRNA levels of CXCL10 were most significantly associated with lobular inflammation [15] while its peripheral levels had the best utility to discriminate patients with advanced fibrosis [16].

Relatively little information is available on the importance of chemokines in acute HCV infection. In four chimpanzees rechallenged with different HCV genotypes after spontaneous clearance of the initial HCV infection, intrahepatic mRNA levels of CXCL10 and CXCL9 were shown to be significantly elevated following the second inoculation [17]. In another study of 10 naïve chimpanzees, intrahepatic mRNA induction of CCL3 was increased in animals that achieved viral clearance compared to those that developed chronic HCV infection [18]. Elevated peripheral levels of CXCL10, CCL4, and CCL5 were also detected in a recent study of patients with acute HCV infection [19].

In the present study we analyzed peripheral expression levels of CXCR3- and CCR5-associated, CCL3-4, chemokines in 9 injection drug users (IDUs) with ten acute HCV infections (8 primary infections and 2 reinfections). Study participants were prospectively followed with frequent assessment of their HCV status through the measurement of HCV antibodies and HCV RNA levels. Plasma samples collected pre- and post-viral acquisition permitted analysis of dynamic changes in chemokine levels in relationship to changes in HCV RNA and ALT levels. A median of 26 data points were analyzed per patient by the use

of recently developed statistical techniques [20–23] that enabled apparent patterns of both long-term and short-term dynamics to be quantified and statistically assessed.

Materials and Methods

Study subjects

Subjects analyzed here were selected from participants in the SWAN Project, which recruits young, high-risk IDUs from streets, parks, and other settings on the Lower East Side of Manhattan through street outreach, needle exchange programs, and word-of-mouth. Eligibility criteria are age 18 to 35 years or initiation of injection drug use within the past 5 years and injection of illicit drugs at least once in the 30 days before enrollment. At enrollment, interviewers administer a standardized questionnaire and blood is drawn for HCV and HIV antibody and HCV RNA testing as described [24]. HCV antibody- and RNA-negative individuals are offered enrollment in a longitudinal study in which HCV RNA testing by the qualitative TMA assay is performed biweekly. A smaller group of antibody-positive, RNA-negative individuals was also followed weekly to detect and study reinfection. After the first assay with detectable HCV RNA, quantitative plasma HCV RNA testing and serum ALT levels were measured weekly for 16 weeks, then biweekly for 8 weeks, and monthly thereafter. ALT testing was performed at the New York-Presbyterian Hospital Laboratory within 24 hours of specimen collection. All acutely HCV-infected study participants identified between the initiation of the SWAN Project in April 2005 and December 2007 who had at least three positive HCV RNA samples were included in this study. The protocol was approved by the Institutional Review Boards of SUNY Downstate College of Medicine and Weill Cornell Medical College and was consistent with the standards established by the Helsinki Declaration of 1975. Written informed consent was obtained from all study participants.

HCV antibody and RNA measurements

HCV antibodies were assayed by second (HCV EIA 2.0, Abbott Laboratories, Abbott Park, IL) and third (HCV 3.0 ELISA and RIBA HCV 3.0, Ortho Clinical Diagnostics, Raritan, NJ) generation tests. The presence of HCV RNA was determined with the discriminatory HCV Aptima TMA assay (developed and marketed by Gen-Probe, San Diego, CA) [25], with the lower limit of detection of 12.1 (95% confidence interval [CI] 11.1–13.2) copies of HCV RNA/mL. Quantitative HCV RNA testing was performed using the Roche Diagnostics Cobas Amplicor HCV Monitor (Quantitative) Test, Version 2.0 or Roche Diagnostics Cobas Taqman HCV (Quantitative) Test. The assay quantifies HCV RNA from 43 to 6.9×10^9 IU/mL, with an overall limit of detection of 18 IU/mL.

Plasma samples

Blood samples were kept at room temperature or 4°C and transported and processed within 24 hours of collection. The plasma was frozen at –80°C and assayed later in batch.

Chemokine measurements

Plasma CXCL9, CXCL10, CXCL11, CCL3, and CCL4 concentrations were measured using commercially available enzyme-linked immunosorbent assay kits (BD OptEIA, BD Biosciences, San Diego, CA for CXCL9 and CXCL10; DuoSet, R&D Systems, Minneapolis, MN for CXCL11, CCL3 and CCL4) according to the manufacturer's protocol. All samples were assayed undiluted in triplicate.

Statistical analysis

We used natural log transformed data for analysis. We modeled the viral-immune processes in each subject on two time-scales: a long-term smooth trend and short-term fluctuations about that trend. This was designed to separate time scales in which a slow increase in the overall level of chemokines is apparent along with strong correlations in fluctuations about that trend.

The long term trend is estimated via recent developments in functional data analysis [20–23]. After removing this trend, short-term fluctuations about it are examined through Spearman's rank correlations. Statistical confidence is evaluated by a mixed continuous-residual bootstrap. We modeled data from 50 days prior to 300 days post infection and transformed all observations using natural logarithms to obtain data that follows the normal distribution.

We used functional data analysis methods designed to account for the unevenness in sampling times. These methods also avoid the imposition of parametric assumptions on the analysis. The data were characterized by variation in the frequency and timing of data points. Although the data are sampled on approximate weekly intervals, missing observations add irregularity to the sampling design. To estimate long term trends for individual subjects, some of whom may have had few data points available, we adapted novel methods described elsewhere [21]. For each marker (HCV RNA, ALT, and chemokines), an average trajectory was measured via a penalized spline smooth of the data pooled across all subjects. The within-subject correlation surface for each marker was then estimated by a bivariate penalized spline. A principal components analysis was taken for the estimated correlation surface and enough components calculated to explain 99% of the estimated total variation. Individual long-term trends were estimated by a ridge regression of the individual marker data on the retained principal components. This methodology allows trajectories for subjects with sparse data to be estimated by borrowing information from subjects with more data that exhibit similar patterns, even when they do not share the same sampling times. The use of ridge regression [26] further reduces the variance in the estimated trajectories.

After the long-term trends were estimated, short-term dynamics were evaluated by calculating Spearman's rank correlation using residuals from that trend. Pairwise correlations were calculated between chemokines, ALT, and HCV RNA measured at the same time. Correlations were also calculated between residuals in the same subject that were within two days of being one week apart and within two days of being two weeks apart.

We investigated in depth a study participant (00105) with a long series of regularly spaced samples. An apparent change-point was estimated by minimizing the variance of HCV RNA on either side. This subject's HCV RNA, ALT and chemokine values were then smoothed to estimate long-term trends, and pairwise correlations for these residuals were examined on either side of the change-point. (See supplementary material for additional details).

Results

Study participants

Chemokine levels were measured on a total of 9 subjects with acute HCV infection with a mean age of 23 years (Table 1). Of these, 6 were men and 3 were women, 8 were Caucasian. Eight study participants had primary acute HCV infections; one participant who had been infected previously, as indicated by HCV seropositivity at enrollment, had acute reinfection. One subject had two HCV infections with at least 6 months and 15 negative HCV RNA samples between the infections. Consequently, we were able to analyze chemokine levels in

nine subjects with a total of ten HCV infections (eight primary infections and two reinfections).

In terms of the outcome, two participants resolved the infection (defined as at least six months of undetectable HCV RNA), one on two occasions. One participant cleared HCV following antiviral therapy, 4 developed chronic HCV infection, and 2 discontinued their participation in the study at weeks 7 and 17 after infection. Per patient, we measured CXCL10 on a mean of 28 (median 26) and CXCL9 and 11 on a mean of 27 (median 26) samples. On average, 23 quantitative HCV RNA measurements (median 18) and 25 ALT measurements (median 19) were available per patient. The average time between samples was 12 days for measurements of CXCR3-associated chemokines, 14 days for measurements of CCR5-associated chemokines, 13 days for ALT, and 10 days for HCV RNA measurements. Time between samples was calculated during the period from 50 days prior to 300 days after viral acquisition, the same time interval used for the statistical analysis

Chemokine measurements

Changes in chemokine, ALT, and HCV RNA levels during acute HCV infection are illustrated in Figure 1. While we observed increases in CXCR3-associated chemokines, neither CCL3 nor CCL4 changed appreciably following infection. Notably, CXCL9-11 induction did not occur early in infection, but was delayed for several weeks in all patients. Chemokine increases were followed by ALT elevation that demonstrated a similar pattern. Moreover, in the first 70 days post infection, we observed a strong similarity in the “sawtooth” patterns between chemokines, ALT and HCV RNA (most visually pronounced in subjects 00059, 00105, 00108). The fold increase for all three CXCR3-associated chemokines appears to be comparable. Both chemokines and ALT decrease 3–4 months post infection. However, among patients with chronic HCV infection, chemokine levels remain elevated compared to their initial values.

The reconstructed mean trajectory illustrates smooth increases in CXCR3-associated chemokines and ALT, peaking between 70 and 85 days (Figure 2). These trajectories are estimated through non-parametric smoothing techniques and are based on 129 observations for HCV RNA, 166 for ALT, 195 for CXCL9, 200 for CXCL10 and 190 for CXCL11. After the chemokine peak, there is a gradual slow decrease that is more gradual than the increase initially observed. We also observe precipitous decrease in HCV RNA levels where quantitative measurements are available. As illustrated in Figure 2, we noted a second peak in HCV RNA. However, while the over-all decline of HCV RNA is supported by the data, the second peak results from the sparse and non-uniform quantitative HCV RNA measurements and is likely to be spurious. CCR5-associated chemokines are not apparently affected by the infection.

The certainty of the estimated reconstructed trajectory can be gleaned from the inspection of the 5 and 95 percentiles of the bootstrapped reconstructed trajectory for each chemokine at each time point (Figure 3). Confidence intervals appear to provide reasonable coverage of the estimated trajectories for subjects both with dense as well as sparse data. The peaks in CXCL9-11 and ALT levels are well resolved for subjects with dense data. Confidence intervals are consistently wider for those with sparse data. As noted previously, no specific patterns are consistently observed for CCL3 and CCL4 reconstructions.

Time to initial chemokine elevation

We examined the time to first elevation in measured CXCL9-11 levels, which we defined as at least a four-fold increase in levels over the average of the pretreatment values.

Participants 00110 and 00257 were excluded from this analysis due to the small number of chemokine measurements during the first few months of the infection. Reinfections in participants 00240 and 00257 were also excluded from this analysis. On average, elevation in CXCL10 levels was first detected 38 days after HCV acquisition (range, 20–61 days). Elevations in CXCL9 and CXCL11 were first detected 52 (range, 27–72) and 53 (range, 27–77) days after infection, respectively. No differences in time to elevation were observed comparing subjects that cleared the infection relative to those that became chronically infected.

Time to peak chemokine levels

The time to maximum ALT and CXCL9-11 levels occurred approximately three months post infection. ALT peaked 75 (95%CI 32–90) days, CXCL10 peaked 72 (95%CI 44–113) days, CXCL9 peaked 83 (95%CI 63–114) days, and CXCL11 peaked 77 (95%CI 63–133) days after the onset of HCV infection. No differences in time to peak elevation were observed comparing subjects that cleared the infection relative to those that became chronically infected.

Correlation between chemokines, HCV RNA, and ALT levels

To investigate the short-term relationship between the chemokines, ALT and HCV RNA, we analyzed the correlation between departures of these parameters from the estimated long-term trends for all study participants utilizing all samples on which chemokine levels were measured. We found a high level of correlation among the CXCR3-associated chemokines (ρ [CXCL9, CXCL10]=0.602, $p<0.001$; ρ [CXCL9, CXCL11]=0.706, $p<0.001$; ρ [CXCL10, CXCL11]=0.428, $p<0.001$). When evaluating the correlations between the chemokines and the virus, we noted the strongest correlation between HCV RNA and CXCL10 ($\rho=0.528$, $p=0.004$). Evaluating the relationships between chemokines and ALT, we noted the strongest correlation between ALT and CXCL9 ($\rho=0.452$, $p=0.008$). For all other correlations between chemokines, HCV RNA, and ALT, ρ was <0.35 or were not significant at the 0.05 level.

Lag correlations for chemokines, HCV RNA, and ALT

We next computed correlations between markers at the current time and at one- and two-week lags. ALT showed positive correlation with all CXCR3-related chemokines measured in the previous week (ρ [CXCL10, ALT]=0.386, $p=0.006$; ρ [CXCL9, ALT]=0.390, $p=0.024$; ρ [CXCL11, ALT]=0.405, $p<0.001$). These findings indicate that an increase in chemokine levels is associated with a subsequent increase in ALT levels. CXCR3-associated chemokines also exhibited negative auto-correlation at one week lags (ρ [CXCL9, CXCL9]= -0.281 , $p=0.050$; ρ [CXCL11, CXCL11]= -0.519 , $p<0.001$; ρ [CXCL11, CXCL9]= -0.266 , $p=0.034$; ρ [CXCL9, CXCL11]= -0.390 , $p=0.014$) suggesting a short-term oscillation. ALT was positively auto-correlated at week 1 ($\rho=0.332$, $p=0.04$) suggesting a possible oscillation at longer intervals. We also noted weak negative correlations between HCV RNA and CXCL9/CXCL11 measured two weeks before (ρ [CXCL9, HCV RNA]= -0.300 , $p=0.018$; ρ [CXCL11, HCV RNA]= -0.267 , $p=0.048$) while weak positive correlations were found between CXCL9 and two weeks following, HCV RNA (ρ [HCV RNA, CXCL9]=0.298, $p<0.042$). Correlations at larger lags beyond two weeks tended to be small.

In depth analysis of one participant with frequent sampling

Subject 00105 provided a particularly long and consistent pattern of weekly samples up to day 540 after HCV acquisition that revealed a qualitative change in the measurements at approximately day 210 (Figure 4A). At this point, HCV RNA levels increased substantially accompanied by a four-fold reduction in the volatility of measurements about the estimated

long term trend ($p < 0.0001$). A similar reduction in volatility was observed in ALT ($p = 0.0039$) and in the CXCR3-associated chemokines (CXCL10: $p < 0.0001$, CXCL9: $p = 0.0002$, CXCL11: $p = 0.0077$). After accounting for the change in volatility, similar patterns of Spearman's rank correlation and lagged correlation were observed on both sides of the change point (Fig. 4B). Correlations prior to the change point agreed well with the correlations observed in the pooled sample data for all study participants as reported above. No non-lagged correlation changed by more than a factor of 2. After the change-point, a consistent decrease was found in the one-week lag between ALT and the CXCR3-associated chemokines $\rho[\text{CXCL10, ALT}] = 0.10$ to -0.23 , $\rho[\text{CXCL9, ALT}] = 0.16$ to -0.15 , $\rho[\text{CXCL11, ALT}] = 0.21$ to -0.06 . The interpretation of correlations of repeated short-term oscillations in chemokines is visually apparent in Figure 4A in which HCV RNA and CXCR3-associated chemokines exhibit tightly-associated "sawtooth" patterns on a 2 to 3 week cycle while similar cycles are somewhat smoother in ALT.

Discussion

In the present study, we describe dynamic changes in CXCR3- and CCR5-associated chemokines and their relationship with HCV RNA and ALT levels during acute HCV infection in nine IDUs. We show that CXCR3-associated chemokines follow a general pattern that develops after viral acquisition. The first substantial elevation in CXCL9-11 is observed three to eleven weeks after the development of detectable HCV RNA, and it is closely followed by elevation in ALT levels. After the initial delayed response, subsequent changes in chemokine levels closely follow the changes in HCV RNA. We also observed a correlation between these chemokines and ALT levels one week later. Negative auto-correlations of chemokine levels at one week lags suggested substantial week-to-week oscillations. In contrast, CCL3 and CCL4 did not vary substantially during acute HCV infection, which suggests that CCR5-associated chemokines most likely do not play an important role in lymphocyte recruitment during this phase of HCV infection.

CXCR3-associated chemokines were previously shown to play an important role in recruiting lymphocytes to the liver during chronic HCV infection [27]. In subjects with chronic infection, immune cells are incapable of HCV eradication, but their accumulation in the liver may support the development of liver fibrosis. These cells, potentially through the production of interferon γ , induce chemokine secretion further promoting intrahepatic inflammation that can lead to the progression of fibrosis and end stage liver disease [14]. To date, very limited data exist on chemokine expression during acute HCV infection. While potentially the virus itself or interferon α/β produced by infected hepatocytes could induce chemokine production, we did not observe elevation of CXCR3-associated chemokines during the first several weeks after viral acquisition. While this delayed induction might be associated with the development of HCV-specific adaptive immunity or natural killer cell activation, additional research is required to verify this association. After the initial delayed elevation in chemokine levels, we observed that each increase in HCV RNA is closely followed by chemokine induction, potentially leading to the influx of immune cells to the liver. Immune-mediated elimination of virally-infected hepatocytes subsequently leads to the release of ALT. We observed ALT elevation after each increase in chemokine levels. In individuals whose immune responses are incapable of viral clearance, the oscillations in HCV RNA, chemokine and ALT levels stabilize, transitioning into chronic infection. This change-point is clearly observed at approximately 210 days post infection in one subject on whom we had frequent and prolonged sampling. Preceding this date, HCV RNA, ALT and CXCR3-associated chemokines are substantially more volatile than after this date. We also observed higher mean HCV RNA levels following this timepoint. Based upon our observations in this subject, these changes may reflect differing functional manifestations of acute versus chronic infection with a transition believed to occur approximately six months

after infection acquisition. This transition between the acute and chronic phases of HCV infection may be similar to that observed in other chronic viral infections such as HIV. On both sides of the change-point, however, a consistent dynamic is apparent with fast recruitment of CXCL10 associated with increases in HCV RNA and following a one-week lag, elevation of ALT.

This study is limited by the small number of participants evaluated, the variability in the number and timing of sample acquisition, and the loss of participants to follow up. Some of these are inherent difficulties when working with high-risk, young IDUs, the population in which 60% of incident HCV infections occur [28]. To overcome these limitations, we used recently developed methods in functional data analysis to make use of the assumed smoothness of parameter trajectories and to borrow strength between subjects in order to obtain a refined picture of chemokine dynamics in acutely-infected HCV subjects. Statistical confidence in our results was carefully assessed through bootstrap methods. These techniques have potential broad application in a wide range of contexts from those with irregularly spaced data with small sample sizes as well as in more regular contexts.

In conclusion, we found that during acute HCV infection, CXCR3-associated chemokines start to increase several weeks after virus acquisition, suggesting a role for cellular immune responses in chemokine induction. Subsequently, dramatic week-to-week oscillations suggest frequent, repeated cycles of gain and loss of immune control during the acute phase of HCV infection. Progression to chronic infection may be marked by an abrupt dampening of these oscillations. To our knowledge, this is the first systematic assessment of dynamic changes in CXCR3- and CCR5-associated chemokines throughout the entire course of acute HCV infection in humans. A recent publication by Stacey *et al.* reported, in agreement with our findings, delayed induction of cytokines after HCV acquisition [19]. However, their primary focus was HIV infection, with HCV-infected individuals included only as controls, and they only evaluated changes in cytokines during the first 20 days after infection. An important but unanswered question is whether chemokine levels during acute HCV infection can differentiate individuals early in infection who are likely to eradicate the virus versus those who are likely to become chronically infected. In our study of nine acutely infected subjects, two spontaneously cleared the infection (one participant on two occasions) and four developed chronic hepatitis C. Comparison of these two groups did not reveal any differences in early chemokine and ALT kinetics, suggesting that chemokine release most likely does not affect the ultimate outcome of the infection. However, the small number of subjects precluded us from drawing final conclusions about this topic. Future studies with larger numbers of subjects and more frequent sampling are needed to precisely define the role of chemokines in determining or predicting the outcome of acute HCV infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

HCV	hepatitis C virus
IFN	interferon
IDU	injection drug user
ALT	alanine aminotransferase
TMA	transcription mediated amplification
CI	confidence interval

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Figure 1. Individual plots illustrating HCV RNA, chemokine, and ALT values for the nine subjects included in the study

Left Y-axis indicates ALT and chemokine levels and right Y-axis indicates HCV RNA levels. Days before and after viral acquisition are indicated along the X-axis. Individual parameters are indicated in the figure legend shown in the upper left panel. Dots along the X-axis represent qualitative HCV RNA results with blue indicating a negative result and red indicating a positive result. Infection outcome is noted in the upper left hand corner above each illustration.

Figure 2. Mean HCV RNA, chemokine, and ALT smoothed trajectories before and after HCV acquisition

Figure 3. Bootstrapped confidence intervals for reconstructed trajectories for subjects with A) dense and B) sparse data

Dashed blue lines indicate 5 and 95 percentiles of the bootstrapped distribution and solid blue line indicates the median bootstrap value. Red lines indicate the estimated reconstructions. Peak levels of CXCR3-associated chemokines are clearly apparent while little trend is noted in CCR5-associated chemokines. Confidence intervals for subjects with sparsely observed data are substantially wider than those obtained from subjects with dense sampling.

Figure 4. Detailed evaluation of subject 00105

Between days 0 and 540 after viral acquisition, subject 00105 had approximately weekly sampling that enabled us to identify A) Change-point, a qualitative change in HCV dynamics at approximately 210 days post infection. Log transformed HCV RNA, chemokines, and ALT levels are plotted and the smoothed trajectories for each parameter are computed as described in Methods. After the change-point, HCV RNA increases substantially with significantly smaller variance of CXCR3-associated chemokines and ALT. B) Spearman's rank correlations at 0, 1 and 2 week lags. For 0-week correlations, quantities along the diagonal, from upper left to lower right, both before and after the change-point, are, by definition, 1.0. Correlations lagged by 0, 1, and 2 weeks in data

obtained before (red) or after (blue) the change-point. The columns represent quantities lagged behind the rows.

Table 1

Patient demographics and infection outcomes

Patient	Age ^a	Gender	Race	Infection	Outcome
00038	19	F	Caucasian	Primary	Resolution upon treatment
00226	24	M	Caucasian	Primary	Unknown
00110	19	F	Caucasian	Primary	Chronic infection
00207	21	M	Caucasian	Primary	Unknown
00257	21	F	Caucasian	Reinfection	Chronic infection
00059	27	M	Caucasian	Primary	Chronic infection
00108	25	M	Caucasian	Primary	Spontaneous resolution
00240	20	M	Mixed	Primary	Spontaneous resolution
00240	21	M	Mixed	Reinfection	Spontaneous resolution
00105	25	M	Caucasian	Primary	Chronic infection

^a Age at time of HCV acquisition