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HCV RNA profiles among chronic HIV/HCV coinfecting individuals in ESPRIT; spontaneous HCV RNA clearance observed in 9 individuals

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Abstract

Objectives: Studies have shown that HCV RNA levels remain stable over time in HIV/HCV coinfecting individuals taking cART, while spontaneous clearance of HCV RNA during the persistent infection phase has been documented only rarely among those with the CC IL28B genotype. This study describes HCV RNA profiles and factors associated with changes over time in HCV RNA levels in the ESPRIT study.

Methods: HIV/HCV coinfecting individuals positive for HCV RNA were included. Follow-up was counted from first HCV RNA positive test and censored at the initiation of interferon-based treatment. HCV RNA and IL-28B measurements were measured in the same reference laboratory. Random effects mixed models were used to analyse changes over time in HCV RNA.

Results: 312 (151 IL-2 arm, 161 control arm) mostly white (89%), male (76%) ESPRIT patients were included with a median of 5 HCV RNA measurements per person (IQR 3-6; range 1-9). Median follow-up was 5 years (IQR: 2 - 6). At baseline 96% were taking cART and 93% had undetectable HIV-RNA. HCV RNA levels decreased 13% per year over the study period (95% CI 8-18%; $P<0.0001$). Baseline HCV RNA levels and the change over time in HCV RNA did not differ by randomisation arm ($P=0.16$ and $P=0.56$, respectively). Nine individuals spontaneously cleared HCV RNA during follow-up (IL-28B genotypes: CC 5 (56%), CT 4 (44%).

Conclusions: HCV RNA levels decreased over time in this population with well-controlled HIV infection. Spontaneous clearance of HCV RNA was documented in 5 individuals with IL28B genotype CC and 4 with the CT genotype.

Introduction

When used together with combination antiretroviral therapy (cART), subcutaneous recombinant interleukin (IL)-2 is known to raise CD4 cell count more than cART alone¹. The ESPRIT trial randomised HIV positive individuals with CD4 cell count above 300 cells/mm³ to receive IL-2 plus antiretroviral therapy or antiretroviral therapy alone, with the aim of comparing the rate of opportunistic disease or death from any cause¹. Although the study concluded that substantial increases in CD4 cell count associated with the administration of IL-2 in addition to cART did not yield any benefit with regards to clinical endpoints¹, other studies have shown that IL-2 may be associated with suppression of HCV RNA in HIV/HCV coinfecting individuals. Small pilot studies have shown that IL-2 treatment for HCV infection may lead to a reduction in liver transaminases, improvement in liver function tests and in some cases sustained suppression of HCV RNA^{2,3}.

Where new direct-acting antivirals are not available due to cost or supply issues, interferon-based treatment for HCV remains the standard of care among HIV/HCV coinfecting individuals⁴. Plasma HCV RNA levels and IL-28B gene variant have been shown to be two of the most important predictors of sustained virological response (SVR) to treatment with pegylated-interferon plus ribavirin^{5,6}. Consequently, it is important to understand which factors are associated with high levels of HCV RNA and which affect changes over time in HCV RNA levels.

One study of 264 HIV/HCV coinfecting individuals actively injecting drugs found that HCV RNA levels increased slowly but significantly over time by approximately 6% each year⁷. Another larger study of 1,541 HIV/HCV coinfecting individuals, which focused on the role of HIV therapy on the course of HCV RNA, found that among those taking cART HCV RNA levels remained stable over time, but that for those not taking cART they increased by 27.6% each year⁸. Further, both studies identified HCV genotype 1 and high levels of HIV RNA as significant predictors of high levels of HCV RNA^{7,8}.

The IL-28B gene, in particular the CC gene variant, has been strongly associated with a superior response to treatment with pegylated-interferon for HCV-monoinfected and HIV/HCV coinfecting individuals and also an increased likelihood of spontaneous clearance of HCV RNA following initial infection⁹. In addition, small observational studies of HIV/HCV coinfecting individuals in the persistent infection phase have identified spontaneous clearance of HCV RNA in a handful of individuals, all with the IL-28B CC gene variant¹⁰. This study aimed to describe the natural history of HCV RNA among HIV/HCV coinfecting individuals in the persistent infection phase in the ESPRIT study, and to consider the effect of IL-2, among other factors, on HCV RNA levels over time.

Methodology

All chronic HIV/HCV coinfecting individuals enrolled in the ESPRIT study, positive for HCV antibody and HCV RNA were included. Baseline was defined as entry to the study, while follow-up was censored at the initiation of interferon-based treatment for HCV or the date of spontaneous clearance of HCV RNA. HCV RNA measurements were taken at study entry and annually during follow-up. A random effects mixed model was used to analyse changes over time in HCV RNA measured on the log₁₀ scale. The covariates adjusted for were ESPRIT randomisation arm (IL-2 or control arm), randomisation date, age, gender, race, study region, injecting drug use, HCV genotype, time from baseline, CD4 cell count nadir, and the time-updating variables HIV RNA, CD4 cell count, hyaluronic acid and cART status (taking cART yes or no, defined as ≥ 3 antiretrovirals from any class). The intercept and time from baseline terms were included as random variables so that separate slopes could be estimated for each individual in the analysis. An unstructured covariance structure was used throughout.

Individuals who spontaneously cleared HCV RNA during the chronic phase of infection were cross-checked with clinical sites to ensure that interferon-based treatment had not been administered and were IL-28B genotyped. All HCV RNA measurements and IL-28B genotyping determinations were performed in the same reference laboratory. HCV RNA measurements were performed using the Versant HCV RNA 3.0 assay by Bayer Diagnostics, which has a lower limit of detection of 615 IU/ml. Baseline and all follow-up samples were tested for each individual. The IL-28B polymorphism test is a polymerase chain reaction analysis of a single nucleotide polymorphism (rs12979860) requiring whole blood.

Results

Three hundred and twelve chronic HIV/HCV coinfecting individuals from ESPRIT were included (151 from the IL-2 arm and 161 from the control arm). The study follow-up period spanned from May 2000 to January 2009 with a median follow-up per individual of 5 years (IQR 3 - 6; Range 1 - 9). A median of 5 (IQR 2 - 6) HCV RNA measurements per individual were included. The randomisation groups were well-matched at baseline (Table 1).

The majority of individuals included were male (IL-2 arm: 78.2% vs. control arm: 73.9%), of white race (88.1% vs. 90.1%) and aged less than 40. A large proportion were infected with HIV via IDU (76.2% vs. 66.5%), but few individuals were also coinfecting with HBV (2.9% vs. 0.7%). The most common HCV genotypes were G1 (58.9% vs. 57.1%) and G3 (19.9% vs. 19.9%). The majority of

individuals were taking cART (96.0% vs. 95.0%) and consequently few had detectable HIV RNA (5.3% vs. 8.7%) and median CD4 cell counts were high (434 vs. 435 cells/mm³).

Factors associated with HCV RNA levels

Overall HCV RNA levels decreased 12.8% per year over the study period (95% CI 7.6 - 17.8%; $P<0.0001$), while 9 individuals spontaneously cleared HCV RNA during the chronic phase of infection. Omitting the 9 individuals who cleared HCV RNA during follow-up HCV RNA levels decreased 11.7% per year (95% CI 6.4 – 16.7%; $P<0.0001$). Baseline levels of HCV RNA were significantly associated with HCV genotype and HIV RNA. HCV genotype 3 was associated with 64.7% lower HCV RNA than HCV genotype 1 (95% CI 43.5 – 77.9%; $P<0.0001$), while undetectable HIV RNA was associated with 20.4% lower HCV RNA compared with HIV viral load above the limit of detection (95% CI 3.7 – 34.2%; $P=0.019$). Older age was also associated with higher HCV RNA with borderline statistical significance (13.8% higher per 5 years older (95% CI -1.3% to 31.4%; $P=0.075$)).

An interaction term between randomisation arm and time from baseline was added to the model to see whether the rate of HCV RNA decline over time was affected by the addition of IL-2. Baseline levels of HCV RNA appeared to be somewhat higher among those in the IL-2 randomisation arm and the rate of decline in HCV RNA over time somewhat faster (Figure 1). However, neither effect approached statistical significance and there was no evidence to suggest that randomisation arm was associated with baseline HCV RNA levels or the rate of decline in HCV RNA over time ($P=0.16$ and $P=0.56$, respectively). Interaction terms including the other covariates were also non-significant (all $P>0.3$, data not shown), indicating that none of the model covariates were significantly associated with the rate of decline in HCV RNA.

Spontaneous HCV RNA clearance and IL-28B genotype

There were 9 cases of spontaneous HCV RNA clearance during the persistent infection phase of HCV infection over the course of the study. The IL-28B genotypes of these individuals were CC: 5/9 (55.6%) and CT: 4 (44.4%), none of them had the TT genotype. The median age of these nine cases was 38 (IQR 36 - 42), 5/9 (55.6%) were male and 8/9 (88.9%) were of white race, the other was Asian. None of these individuals were HBsAg positive; although for one of them HBsAg status was unknown. The HCV genotype distribution of these individuals was G1: 3/9 (33.3%), G2: 1/9 (11.1%) and G3: 5/9 (55.6%), while median baseline HCV RNA was 6.01 Log₁₀ IU/ml (IQR 4.60 – 6.13). The median duration of follow-up prior to becoming negative for HCV RNA was 24 months (IQR 12 – 72; range 12 - 84). The median nadir CD4 cell count of these cases was 199 cells/mm³ (IQR 155 - 352),

median CD4 cell count at spontaneous clearance was 435 cells/mm³ (IQR 400 - 702), further HIV RNA was undetectable for 5/9 (55.6%).

Discussion

This study of chronically infected HIV/HCV coinfecting individuals documented a significant decline in HCV RNA levels over time. This finding is in agreement with a recent study of small number of HIV/HCV coinfecting individuals enrolled in a cART initiation trial¹¹. The authors found that a transient increase in HCV RNA may result from initiation of cART, however, in the long term upregulation of CD4, CD8 and alpha-beta T-cell activity was demonstrated to lead to a decline in HCV RNA¹¹. Other studies of the natural history of HCV RNA have tended to suggest that HCV RNA levels remain fairly stable over time^{7,8}. One of the strengths of this study is that all HCV RNA measurements were determined in the same reference laboratory, which is not always the case in many observational settings, and could be a contributor to the contradictory findings of this study and others looking at the natural history of HCV RNA. However, this study differs from previous studies in that it included individuals enrolled in a clinical trial who had very well-controlled HIV infection. At baseline >95% of the 312 individuals included in the study were taking cART and only 7.1% had detectable HIV RNA. In addition, detectable HIV RNA was associated with significantly higher levels of HCV RNA, which confirms the findings of previous studies and suggests that control of HIV RNA following cART can lead to an improved cellular response to HCV^{8,12}.

HCV genotype 1 was associated with higher levels of HCV RNA compared to other genotypes, in particular HCV genotype 3. This finding is in agreement with other studies of the topic and, while high levels of HCV RNA are associated with a poor response to treatment with pegylated-interferon, may help to explain the comparatively low rate of SVR seen for these treatments in HCV genotype 1^{6-8,13}. The design of this study allowed for direct comparison of HCV RNA profiles among HIV/HCV coinfecting individuals receiving cART alone and IL-2 in addition to cART. While treatment with IL-2 has previously been associated with an improvement in liver function and suppression of HCV RNA in some cases^{2,3}, this study found no evidence to suggest that the addition of IL-2 had any long-term effect on the course of HCV RNA. However, IL-2 was administered twice daily for 5-days on an 8-week cycle, while HCV RNA measurements were taken annually. Therefore, we might not have been able to detect the effect of IL-2 on HCV RNA levels if the effect was only transient.

The IL-28B CC gene variant is known to be associated with a favourable response to interferon-based treatments for HCV and an increased likelihood of spontaneous clearance following initial HCV infection^{9,14}. Further, in studies of HIV/HCV coinfection where a small number of individuals with the CC gene variant have spontaneously cleared HCV RNA during the persistent infection phase, it has been suggested that immune reconstitution as a result of successful cART may lead to the

resumption of the mechanisms responsible for clearance of HCV RNA^{10;14}. This study documented HCV RNA spontaneous clearance during the persistent infection phase in 5 individuals with the CC gene variant and 4 individuals with the CT gene variant. This is the first study to document spontaneous clearance of HCV RNA during the persistent infection phase in individuals with the CT IL-28B gene variant, which suggests that the spontaneous HCV RNA clearance phenomenon during persistent infection is not restricted to the CC gene variant.

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Table 1 Baseline characteristics of HIV/HCV coinfecting individuals in ESPRIT stratified by randomisation arm

Baseline characteristics % / Median (IQR)		IL-2 Arm (N=151)	Control Arm (N=161)	P-value
Male		78.2	73.9	0.38
Age		38 (34 – 41)	39 (34 – 43)	0.21
Race	White	88.1	90.1	0.57
	Non-white	11.9	9.9	
HIV transmission via IDU		76.2	66.5	0.059
HBsAg +		2.9	0.7	0.15
HCV genotype	1	58.9	57.1	0.78
	2	1.3	1.9	
	3	19.9	19.9	
	4	13.3	16.8	
	5	0.7	0	
	Unknown	6.0	4.4	
CD4 cell count		434 (370 - 540)	435 (365 - 553)	0.92
CD4 cell count nadir		163 (70 - 262)	150 (70 - 258)	0.93
Taking cART		96.0	95.0	0.67
Detectable HIV-RNA (>50 copies/ml)		5.3	8.7	0.24
Hyaluronic acid (ng/ml)		35.4 (16.6 – 57.9)	32.1 (18.9 – 65.2)	0.86
Baseline date		AUG/01 (FEB/02 – OCT/02)	AUG/01 (FEB/02 – SEP/02)	0.94

Figure 1 The effect of ESPRIT randomisation arm on the course of HCV RNA levels

