Hepatitis C virus reinfection after sustained virological response in HIV-infected patients with chronic hepatitis C

Running title: HCV reinfection in HIV-infected patients.

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Abstract

Objectives: To assess the incidence of hepatitis C virus (HCV) reinfections after therapy-induced clearance in HIV-coinfected patients with prior chronic hepatitis C.

Methods: Eighty-four HIV-infected subjects, who had previously achieved sustained virological response (SVR) after being treated of chronic hepatitis C, were analyzed. In all of them, at least yearly HCV RNA determinations were carried out during a median (range) of 34 (12-146) months.

Results: Seventy-two (86%) subjects had been people who inject drugs (PWID), of whom 11 (15%) continued to use snorted or injected drugs during the follow-up. Four (4.76%) patients showed HCV reinfection (incidence 1.21 [95% Confidence Interval: 0.3-3.09) cases per 100 person-years]. These patients maintained risk factors for HCV infection. In three cases, HCV genotype switched. Phylogenetic analysis of the remaining case suggested reinfection from his sexual partner.

Conclusion: The incidence of HCV reinfection in the overall population of HIV-coinfected patients who achieved SVR after being treated against chronic hepatitis C is low. A low frequency of risk behavior is the main factor accounting for this modest rate of reinfection. The possibility of reinfection should not be considered a reason against treatment of HCV infection with direct acting antivirals in PWID.

Key words: Hepatitis C virus, HIV, reinfection, parenteral drug use, direct acting antivirals.

Introduction

Hepatitis C virus (HCV) reinfection may occur after spontaneous or treatment-induced clearance of a prior episode of HCV infection [1,2]. HCV reinfections are a major problem in the field of HCV infection. Indeed, the emergence of HCV reinfection episodes raises questions on the durability and effectiveness of immune response against HCV, and, consequently on the potential efficacy of vaccines against HCV. Thus, understanding the mechanisms underlying clearance of and reinfections with HCV is critical to improve our knowledge on the susceptibility to HCV infection and for vaccine design [1,3]. In addition, the chance of reinfections has led some clinicians to be reluctant to treat hepatitis C in specific settings where reinfections might be particularly common, such as in people who inject drugs (PWID) [4]. However, in many PWID are treated against chronic hepatitis C when they have stopped using injected drugs or are under opiate substitution therapy, and in these cases the incidence of reinfection could be completely different. Indeed, in our area, as in others, most of these people either permanently stop using opiates and cocaine or, in case of relapse, they use smoked crack and or heroin [5].

After spontaneous clearance, the reported rates of HCV reinfection in PWID have been extremely variable, ranging from 0.8 to 24.6 per 100 person-years [2,6-11], which has been explained on the basis of differences in risk behaviors for infection between populations. However, these studies included few or no HIV-coinfected subject. In HIV-infected men who have sex with men (MSM), HCV reinfections are particularly common. Thus, rates of reinfections of 9.6 per 100 person-years in patients who had

reached sustained virological response (SVR) after therapy and 4.5 per 100 person-years in those who spontaneously cleared HCV have been described [12], although higher figures have also been reported [13]. In some case series, up to one fourth of patients who cleared HCV after an episode of acute hepatitis C suffered from a reinfection [14]. However, data on the frequency of HCV reinfections in HIV-coinfected PWID, particularly in the setting of subjects who attained SVR after therapy for chronic hepatitis C are scarce. Obtaining information on this issue is critical, as the cost of the newer therapies against HCV require prioritizing potential candidates according to the likelihood of achieving definitive cure of hepatitis C in specific subsets.

The objective of the present study was to assess the incidence and main features of HCV reinfections after therapy-induced clearance in a HIV-coinfected population, mainly made up of PWID.

Methods

Patients and follow-up

From a cohort of HIV/HCV-coinfected patients who received treatment against chronic hepatitis C in two Spanish hospitals and who were prospectively followed from 2001 to 2013, those fulfilling the following criteria were selected for this retrospective analysis: i) Having attained SVR, as defined as undetectable plasma HCV RNA 24 weeks after therapy completion, and, ii) having at least yearly subsequent plasma HCV RNA determinations or plasma samples frozen at -80ºC which enabled such determinations. All patients underwent clinical evaluations at least every six months during the follow-up. In every visit, routine laboratory determinations, including liver function tests were performed.

Definition criteria

When a patient, after developing SVR, tested positive for plasma HCV RNA, a definite diagnosis of reinfection was established if the HCV genotype detected in such an episode was different than the one found in the prior infection. In case the first and the second genotype were the same, the diagnosis of reinfection was done according to the phylogenetic analysis of the strains at the first and at the second episode.

Laboratory procedures

HCV RNA was screened by means of COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 (Roche Diagnostic Systems Inc, Pleasanton, CA, USA) with a limit of detection of 15 UI/ml. When positive, quantification was carried out by

COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test, v2.0 (Roche Diagnostic Systems Inc, Pleasanton, CA, USA).

For HCV genotyping, the core region was amplified by nested PCR as previously described [15,16] except for genotype 3a, which was amplified by semi-nested PCR with the same conditions. The primers used for genotype 3a were: 5'-GTCTGCGGAACCGGTGAGTA [17] and 5'-GGAGGTCTCGTAGACCGTGCA [15], as forward primers for the first and second PCR rounds, respectively, and 5'-TGTCACTGGGGGTCCAGCAC, as reverse primer for both rounds. The PCR products were bi-directionally sequenced using standard capillary electrophoresis techniques.

To determine genetic relatedness between HCV strains, phylogenetic trees were built. With this purpose, sequences were aligned with 10 HCV-genotype 1a (HCV-1a) core consensus sequences from Spanish patients (Genbank accession numbers **KF060663**, **KF060665**, **KF060668**, **KF060670**, **KF060672**, **KF060684**, **KJ739746**, **KJ739756**, **KJ739757**, **KJ739763**) [16,18] using the ClustalW2 software (www.ebi.ac.uk/Tools/msa/clustalw2/). In this alignment the HCV-1a H77 reference sequence (Genbank accession number: **AF009606**) was also included, as well as additional references Core sequences from other HCV genotypes (1b, 1c, 2a, 3a, 4a, 5a and 6a, Genbank accession numbers: **D90208**, **D14853**, **AY746460**, **D17763**, **Y11604**, **Y13184**, **Y12083**, respectively) as outlier sequences. The first 410 base pair positions of the core region for each sample were used in the phylogenetic analyses. These analyses were conducted in MEGA6 software [19]. Genetic distances were estimated by Kimura two parameter model and phylogenetic reconstruction was performed using the Maximum Likelihood method based on Kimura two parameter model with a discrete

gamma distribution and 200 replicates in the bootstrap resampling analysis. FigTree software (tree.bio.ed.ac.uk/software/figtree/) was used to create the final phylogenetic tree.

Statistical analysis

In this study, only a descriptive statistical analysis was conducted. The categorical variables are shown as number (percentage [95% confidence interval –CI-]) and the continuous ones as median (quartile 1-quartile 3). The statistical analysis was carried out using the SPSS statistical software package release 22.0 (IBM SPSS Inc, Chicago, IL, USA) and the Stata 12.0 package (StataCorp, College Station, TX, USA).

Ethical issues

The study was designed and conducted following the Helsinki declaration. The Ethics Committee of the Hospital Universitario de Valme approved the study. All patients provided informed consent to participate.

Results

Study population

Two hundred and fifty-two HIV-infected patients were given therapy against chronic hepatitis C at the participant institutions during the study period. One hundred and eleven (44%) of them achieved SVR. However, 27 subjects were not included in the present analysis because they neither had subsequent yearly plasma HCV RNA determinations nor available frozen samples to determine them. Namely, nine of these patients had a follow-up shorter than 1 year after SVR, six were lost to follow-up and in 12 previously available samples had run out. Therefore, 84 patients were included in this analysis. Twenty-five (93%) out of 27 patients not included in the study had been formerly PWID and six (24%) of them were on opioid substitution therapy. One (4%) patient of those who were not included continued to use smoked cocaine or heroin.

All subjects were Caucasian. The mean (range) follow-up after SVR was 34 (12-146) months. Most patients were prior PWID and had attained SVR after a course of pegylated interferon plus ribavirin therapy. The main features of the population studied are displayed in Table 1. During the follow up, 15 out of 72 former PWID (21%) were on methadone maintenance therapy. Seven of them, as well as four further of those 72 individuals (15%), also reported to use cocaine and/or heroin, in most cases by smoking or snorting, but occasionally by unsterile injection. The remaining 53 subjects who had become infected with HCV through drug injection did not report to use any opiate or cocaine during the follow-up.

Reinfection episodes

Four [4.76% (95% CI: 1.31-11.74] patients tested positive for HCV RNA after having attained SVR. The specific characteristics of these patients are shown in Table 2. HCV genotype switched from the primary infection to the reinfection in three patients. They were PWID, one of them on methadone maintenance therapy, who intermittently injected drugs during the follow-up. The remaining subject (patient 4) carried HCV 1a in both the first and the second episode. He was a MSM who presented with asthenia and jaundice 80 months after having achieved SVR. At this time, he had only one steady sex partner, who was also infected with HCV genotype 1a and they reported unprotected anal sex. The phylogenetic analyses (Figure 1) showed a closer genetic distance between the strain from the patient at the second episode and the one from his sexual partner taken at the same date (0.007; standard deviation [SD]=0.004) than between the samples from the patient collected in the first and in the second episode (0.015; SD=0.006). This fact was considered as highly suggestive of reinfection, the partner being the source.

The incidence of reinfection was 1.21 (95% CI: 0.3-3.09) cases per 100 person-years (Figure 2). Just one case was symptomatic. This patient was given triple therapy with pegylated interferon plus ribavirin plus telaprevir for 12 weeks during the acute phase of the disease, reaching again SVR. The remaining cases remained untreated and evolved to chronic hepatitis C.

Three out of 11 (27%) patients who used heroin and/or cocaine during the follow became reinfected. The incidence of reinfection in this particular subset was 8.72 (4.8-23.7) cases per 100 person-years (Figure 2).

Discussion

According to the results of this study, the incidence of HCV reinfection in HIV-coinfected patients with prior chronic hepatitis C who had achieved SVR is low. As the population analyzed herein was mainly made up of former PWID, these results strongly support that the possibility of reinfection should not be a reason against treating these patients with the newer direct-acting antivirals, at least if they have achieved opiate abstinence or are under opiate substitution therapy. In contrast, in subjects with ongoing drug injection, the risk of reinfection is much higher. This fact reinforces the need of implementing measurements to prevent HCV infection in this subset, such as promoting changes in the way of drug administration, opiate substitution therapy or providing individual sterile injection equipment. This is particularly important in subjects who urgently require therapy against chronic hepatitis C, as those bearing liver cirrhosis.

Few data are available on the risk of reinfection after having achieved SVR in HIV-coinfected patients who received therapy against HCV due to chronic hepatitis C. Studies conducted in HIV-infected MSM, in most cases following acute hepatitis C, showed a high incidence of reinfection [12,14], with some subjects developing up to four consecutive episodes [14]. The figures reported in these populations are higher than those found in subjects without HIV infection in other studies, although the latter population included mainly PWID [2,6]. In addition, in studies in which both HIV-infected and HIV-uninfected PWID were included, HIV coinfection turned out to be associated with a higher incidence of reinfection [6,20]. Altogether, these results might lead one to hypothesize that HIV-infected patients are more prone to HCV reinfection.

The inappropriate immune response against HCV observed in HIV infection [21] could underlie this fact. However, the results of the present study suggest that incidence of reinfection mostly depends on the risk behaviors for HCV infection of the specific population. HIV-infected MSM with a high incidence of HCV reinfections usually show ongoing high risk sexual behaviors, such as unprotected anal intercourse, permucosal traumatic sexual techniques, group sex and common sexually transmitted infections causing genital ulcers [12,14,22]. Conversely, in this study, former PWID, who had previously stopped using drugs or who were on opiate substitution therapy, was the predominant population included, as most PWID treated against hepatitis C in our area meet these conditions. And, in our area, a vast majority of these patients do not use parenteral heroin or cocaine anymore. However, 11 subjects reported to use drugs during the follow up, in most cases by smoking or snorting –practices also linked to HCV transmission [23]-, and in this population with ongoing risk behavior the incidence of reinfection was remarkably high. Similarly, in studies where HIV-negative PWID with active drug injection were included, much higher rates of reinfection have been reported [9,11,20], which support that the incidence of HCV reinfections is driven by ongoing risk behaviors to a greater extent than by HIV coinfection. In any case, studies aimed to further clarify the impact of HIV infection on the risk of HCV reinfection should be undertaken.

The reemergence of HCV viremia without HCV sequence switching later than six months after achieving SVR has been considered by some authors as late relapses rather than reinfections [24]. However, subjects with ongoing risk behaviors, who are exposed to the same infection source, e.g. steady sexual couples, might become reinfected with the same viral strain, making differentiation between relapse and reinfection quite

difficult. In this study, cases 1, 2 and 3 were clearly reinfections, as HCV emerged from 36 to 75 months after SVR, with multiple HCV PCR negative results in the meantime, subjects had intermittent risk behaviors and HCV genotype switched. In the fourth case, who also had risk factors, HCV genotype was the same. Although HCV compartmentalization is a proven fact (25), and this has been proposed as a potential mechanism for lower response to treatment with pegylated interferon plus ribavirin in HIV coinfected patients (26), there is no study that has firmly shown that reservoirs could be a cause of re-emergence of HCV beyond 6 months of completing therapy in subjects attaining SVR. In any case, the strain in this patient was closer to that of his sexual partner than to the one observed in the primary infection episode. Moreover, the length of time elapsed after SVR was very long and risk factors persisted. All these reasons support this case was a true reinfection rather than a late relapse.

This study has a few limitations. First, the design was retrospective and patients with samples taken up to one year apart were included. Some studies have suggested that the more frequent the HCV RNA determinations are, the more commonly HCV viremia is found, because some episodes of HCV emergence, which spontaneously clear in a few weeks, would go unnoticed otherwise [27]. However, whereas cure without therapy seems to be more common in subjects with prior spontaneous clearance than in primary infection [1,2], most reinfection episodes appearing after treatment-induced clearance evolve to chronic infections [12,14]. Accordingly, it is unlikely that a significant number of reinfections had not been detected in this study. A second limitation is that population sequencing has been used here for genotyping. Indeed, this procedure is unable to identify minority variants present at a frequency below 20%-30%. Consequently, mixed infections, which could be common in PWID [28,29], appearing

within six months following the end of treatment might have been considered as relapses of primary infection, instead of true reinfections [2]. To what extent the incidence of reinfection would increase if HCV strains emerged in cases considered as relapses in the cohort where the study patients came from had been sequenced using more sensitive procedures would require further studies. Conversely, some authors believe that Sanger sequencing would overestimate the incidence of reinfections, as the emergence of new viral strains might be associated with preexisting minority variants, which become dominant after relapsing, rather than with actual reinfections. These cases would only be proven if high-sensitivity procedures, such as pyrosequencing, are implemented [30]. In any case, the long period between SVR and the emergence of HCV in our patients strongly argues against the possibility of preexisting strain recurrence. A third limitation is that drug use in our study was self-reported. Thus, we cannot rule out some degree of underreporting, particularly in subjects using parenteral drugs intermittently. If so, the main conclusion of this study would be similar, as the incidence of HCV reinfection in the overall population of HIV-infected patients who achieve SVR following treatment of chronic hepatitis C would be low, even with a risk of infection somewhat higher than that considered here. Finally, although the sample analyzed in this study is smaller than that included in others (11,20), this is one of the largest population of HIV-infected patients, including mainly subjects who had been PWID, with SVR, in whom the risk of reinfection has been analyzed so far. In addition, as the follow-up is long, the study population allows determining the incidence of reinfection with a relatively narrow confidence interval. Thus, even assuming the upper margin of the confidence interval (3.09 cases per 100 person-years) this figure would continue to be lower than in other studies.

In conclusion, the incidence of HCV reinfections in HIV-infected patients who achieved SVR following treatment against chronic hepatitis C is low. The existence of ongoing risk behaviors is the main determinant of the chances of reinfection. Because of this, in subjects with continuing risk factors, sequential determinations of plasma HCV-RNA load are warranted. As it is clear that protective immunity is not generated after an episode of hepatitis C, avoiding risk behaviors, such us sharing drug use devices or unsafe sex, is the best way to reduce the risk of reinfections. However, as with HCV infection in general, reinfections will be only eradicated by universal HCV carrier detection and direct acting antiviral-based therapy in all of them.

Acknowledgments

This work was supported by the grants from the Consejería de Salud de la Junta de Andalucía (PI-0335-2013), the ISCIII-Subdirección General de Evaluación and the Fondo Europeo de Desarrollo (ISCIII-RETIC RD06/006 and RD12/0017). KN is the recipient of a Miguel Servet research grant from the Instituto de Salud Carlos III (grant number CP13/00187). JM is the recipient of a grant from the Servicio Andaluz de Salud de la Junta de Andalucía (B-0037). JAP has received a research extension grant from the Programa de Intensificación de la Actividad de Investigación del Servicio Nacional de Salud Carlos III (I3SNS).

Conflict of interest

JAP reports having received consulting fees from GlaxoSmithKline, Bristol- Myers Squibb, Abbot, Gilead and Boehringer Ingelheim Pharmaceuticals, Janssen, VIIH and Pfizer. He has received research support from GlaxoSmithKline, Roche, Bristol-Myers Squibb, Schering-Plough, Abbot, Boehringer Ingelheim Pharmaceuticals, Janssen and Pfizer, lecture fees from GlaxoSmithKline, Roche, Abbot, Bristol-Myers Squibb, Boehringer Ingelheim, Schering-Plough Pharmaceuticals, Janssen, VIIV and Gilead, and payment for manuscript preparation from Bristol-Myers Squibb, Boehringer Ingelheim and Roche, and for development of educational presentations from Gilead. FG has received consulting fees from Abbvie, Merck, ViiV, GlaxoSmithKline and Gilead. KN has received lecture fees from Janssen-Cilag, Roche, Bristol-Meyers Squibb and Merck Sharp & Dohme and has received research support from Janssen-Cilag, Bristol-Meyers Squibb, Merck Sharp & Dohme, Gilead Sciences and Abbott Pharmaceuticals. JM has been an investigator in clinical trials supported by Roche,

Bristol-Myers Squibb and Abbott Pharmaceuticals. He has received lectures fees from Roche, Gilead, Boehringer Ingelheim and Bristol-Myers Squibb, and consulting fees from Boehringer Ingelheim, Bristol Myers-Squibb and Merck Sharp & Dome.

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Table 1. Characteristics of the study population at the time of sustained virological response (n=84).

Parameter	**Value (%)**
Male gender, n (%)	68 (81)
Age*, years	44 (39-47)
Risk factor for HCV infection, n (%)	
Intravenous drug use	72 (86)
Sexual transmission	3 (4)
Unknown	9 (11)
CD4 cell counts*, cells/μL	455 (315-721)
Undetectable HIV RNA, n (%)	73 (87)
IL 28B genotype†	
CC	28 (41)
CT	27 (39)
TT	14 (20)
HCV genotype in prior infection	
1	34 (40)
2	1 (1)
3	39 (46)
4	10 (12)
Prior HCV therapy	
Peg-IFN +RBV¶	73 (88)
Peg-IFN + RBV + DAA§	11 (13)
Liver stiffness*, KPa	6.7 (5.7-8.8)

1 *Median (quartile 1-quartile 3); †Available in 69 subjects; ¶Pegylated interferon plus
2 ribavirin § Pegylated interferon plus ribavirin plus one direct acting antiviral.

Table 2. Main features of patients with HCV reinfection.

Patient No	Risk factor	Months from SVR* to reinfection	Genotype at prior infection	Genotype at reinfection	IL28B Genotype	Maximum ALT† value during reinfection (IU/mL)	CD4 cell count at reinfection	Outcome
1	PWID¶	75	3a	1a	CC	50	535	Chronic hepatitis C
2	PWID	61	3a	4a/c/d	Not available	574	280	Chronic hepatitis C
3	PWID	36	1a	4d	CT	379	494	Chronic hepatitis C
4	MSM‡	80	1a	1a	CC	1976	437	PR + TVR§. SVR.

*SVR: Sustained virological response; †ALT: Alanine aminotransferase; ¶PWID: People who injected drugs; ‡MSM: Man who have sex with men; §Treated with pegylated interferon plus ribavirin plus telaprevir.

Figure 1. Phylogenetic analysis of 22 HCV core region sequences by maximum likelihood method. Phylogenetic tree was built using 10 HCV subgenotype 1a core sequences (named with their genbank accession number), 8 core references sequences (named with the corresponding subgenotype and their genbank accession number), two samples from patient number 4 (P4), one collected in 2006, at the primary infection episode (P4_2006) and the other at the reinfection (P4_2013), and the remaining two from his sexual partner (Partner of patient 4: PP4), one taken at 2005 (PP4_2005), before they were engaged as a couple, and the other at the time of the patient reinfection (PP4_2013). Samples of Patient 4 (P4_2013) and his sexual partner (PP4_2013) collected at the time of the reinfection cluster together branching in the same node with a bootstrap value of 0.56, closer that samples from the patients at the primary infection and at the reinfection. This suggests that the partner was the source of infection.

Figure 2.- Incidence of HCV reinfection in the overall population and in the subset of ongoing drug users. Bars represent incidence (number of cases/100 person-years) with the upper limit of the 95% confidence interval.