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ORIGINAL ARTICLE

Longitudinal evaluation of hepatitis C viral persistence in HIV-infected patients with spontaneous hepatitis C clearance

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Abstract Hepatitis C virus (HCV) viral persistence in patients with spontaneous viral clearance is controversial. Several studies have shown HCV-RNA in peripheral blood mononuclear cells (PBMCs) and/or liver tissue among patients who have cleared the virus spontaneously, suggesting that viral persistence is a common situation that could involve the entire population studied. Thus, our aim was to evaluate HCV-RNA persistence in PBMCs and hepatocytes in subjects infected with the human immunodeficiency virus (HIV). A total of 1508 patients were prospectively followed and tested for anti-HCV antibodies and HCV-RNA to identify the patients who achieved spontaneous viral clearance. In all of the patients, the persistence of HCV-RNA in PBMCs was evaluated longitudinally during 2 years of follow-up. Fifty-nine patients fulfilled the inclusion/exclusion criteria and were included in the study. HCV-RNA was not detected in the PBMCs at baseline [59 PBMCs samples tested; 0 %; 95 % confidence interval (CI): 0–3.3 %] or during the follow-up (147 PBMCs samples tested; 0 %; 95 % CI: 0–2.02 %). Our study shows that HCV viral persistence is not a frequent occurrence in HIV-infected patients who have spontaneously resolved an HCV infection. Thus, the lack of serum HCV-RNA should continue to be addressed as the standard of healing.

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Introduction

Hepatitis C virus (HCV) sustained viral clearance due to a spontaneous resolution or a successful treatment course, characterized by the absence of serum HCV-RNA, is correlated with infection healing [1]. In several studies, a low titer of viral persistence has been found in the peripheral blood mononuclear cells (PBMCs) and hepatocytes in patients with sustained viral clearance [2–5]. In this situation, the presence of residual viremia might have clinical consequences in patients with a resolved HCV infection. Firstly, patients with occult persistence of viremia might be potentially infectious. Secondly, the confirmation of HCV persistence in patients with HCV viral clearance could modify the gold standard of health care: undetectable serum HCV-RNA. However, viral persistence in these patients remains controversial [6, 7].

HCV spontaneous clearance is strongly related with the immunological response; theoretically, viral persistence could occur in an impaired immunological situation [8]. Thus, HCV-RNA persistence at low levels in PBMCs and/or hepatocytes might be more frequent in an immunocompromised population, such patients infected with the human immunodeficiency virus (HIV). Therefore, HIV infection could constitute a suitable scenario for exploring hepatitis C viral persistence in patients with HCV spontaneous clearance.

Methods

Study population

HIV-1-infected patients were included in this prospective longitudinal study. From December 2009 to November 2013, 1508 patients were prospectively followed in two hospitals in southern Spain. Patients who fulfilled the following criteria

were enrolled in the study: (i) presence of antibodies for HCV using enzyme immunoassay (EIA) and confirmed by a recombinant immunoblot assay; (ii) negative serum HCV-RNA (detection limit set at 15 IU/mL); (iii) not treated for acute or chronic HCV infection; and (iv) no hepatitis B virus co-infection. The date of HCV spontaneous clearance was estimated based on the risk factors of HCV infection.

Baseline examinations and follow-up

For patients who met the inclusion criteria, a clinical examination, routine hematological, biochemical, immunological, and virological assessments, and a liver stiffness examination (FibroScan™, Echosens, Paris) were performed. Those patients with negative HCV-RNA in PBMCs were followed up for 2 years. Biannual HCV-RNA determinations in the serum and PBMCs were performed.

HCV-RNA evaluation

The PBMCs from patients were purified from 10 mL of whole blood by centrifugation in Ficoll gradient. The serum and PBMCs samples were conserved in the RNAlater reagent (Ambion, Austin, TX, USA) at −80 °C. The total RNA was isolated from 1 mL of serum or PBMCs using the TriPure Reagent (Roche, Basel, Switzerland). After precipitation, the RNA pellet was dissolved in 10 μL of diethyl pyrocarbonate (DEPC)-treated water, and the RNA concentrations were determined by spectrophotometry. The RNAs were diluted to 1 μg/μL. Reverse transcription of RNA was performed using the SuperScript III system (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The quality of each serum or cellular cDNA sample was evaluated by β-Actin TaqMan Real-Time PCR (catalog number: 4333762T; Applied Biosystems, Foster City, CA, USA). The detection of the 5′UTR of the viral RNA strands was by nested reverse transcription polymerase chain reaction (RT-PCR) using two different primer sets (detailed in Fig. 1) and a common TaqMan probe (5′FAMCCGCAGACCACTATGGCTC3′ BHQ1). The first PCR round included the primers previously described: HCV IO_1 5′CTTCACGCRGAAAGCGYCTA3′ and HCV IO_2 5′CAAGCACCCTATCAGGCAGT3′ [9]. The second round included the primers: HCV IO_3 5′ GCGTTAGTAYGAGTGTYG3′ and HCV IO_4 CR-ATTCCGGTGTACTCAC. The PCR was performed in a final volume of 12.5 μL, with 6.25 μL of QuantiMix Easy Probes Master Mix (Biotools, Madrid, Spain), 1 μL of cDNA, 100 pmol of each primer, 8 μM of the TaqMan probe, and 2 μL of water. The cycling conditions were: 95 °C for 10 min and 45 cycles at 95 °C for 20 s, 54 °C for 30 s, and 60 °C for 20 s.

Ethical statement

The study was designed and performed according to the Helsinki Declaration and was approved by the ethics committee of the Reina Sofía University Hospital, Cordoba, Spain. All of the patients provided a written informed consent form before participating in the study.

Results

Study population

Of the patients included in the cohort, 881 (58.4 %) were confirmed to be seropositive for HCV. Of them, 88 patients (9.9 %) tested negative for serum HCV-RNA without having received anti-HCV treatment. Fifty-nine patients agreed to participate in the study and constituted the study population. The main patient characteristics are summarized in Table 1.

Evaluation of viral persistence in PBMCs

HCV-RNA in serum and PBMCs was measured in all of the patients at baseline. No samples were positive [0 %; 95 % confidence interval (CI): 0–3.3 %]. During the study follow-up, a total of 147 PBMCs samples were evaluated (2.67 samples per patient). Intra-PBMCs HCV-RNA was not detected in any of the samples (0 %; 95 % CI: 0–2.02 %).

Discussion

Instead of viral clearance due to a successful treatment implementation or host-mediated immune response strongly correlated with HCV healing, low titer viral persistence remains a controversial point [2–7]. Several studies have shown HCV-RNA in PBMCs and/or liver tissue among these patients, suggesting that this viral persistence could be a common situation [2, 3]. Nevertheless, other studies have shown that HCV aviremic subjects showed a complete PBMCs HCV clearance, suggesting that PBMCs do not serve as long-term viral reservoirs [6, 7]. Our study is the first to perform a longitudinal prospective analysis of HCV viral persistence in an immunocompromised population, such HIV individuals, who have spontaneously cleared HCV. During the entire follow-up period, PBMCs HCV-RNA was not detected. Our findings demonstrate that PBMCs viral persistence might be considered a rare event in HIV-infected patients who have experienced an HCV spontaneous clearance. The longitudinal design of the study was important because it facilitated the detection of a transitory or intermittent viral presence [9]. Furthermore, in order to reduce the false-negative rate, we included quality control of cDNA and a new set of degenerate oligonucleotides

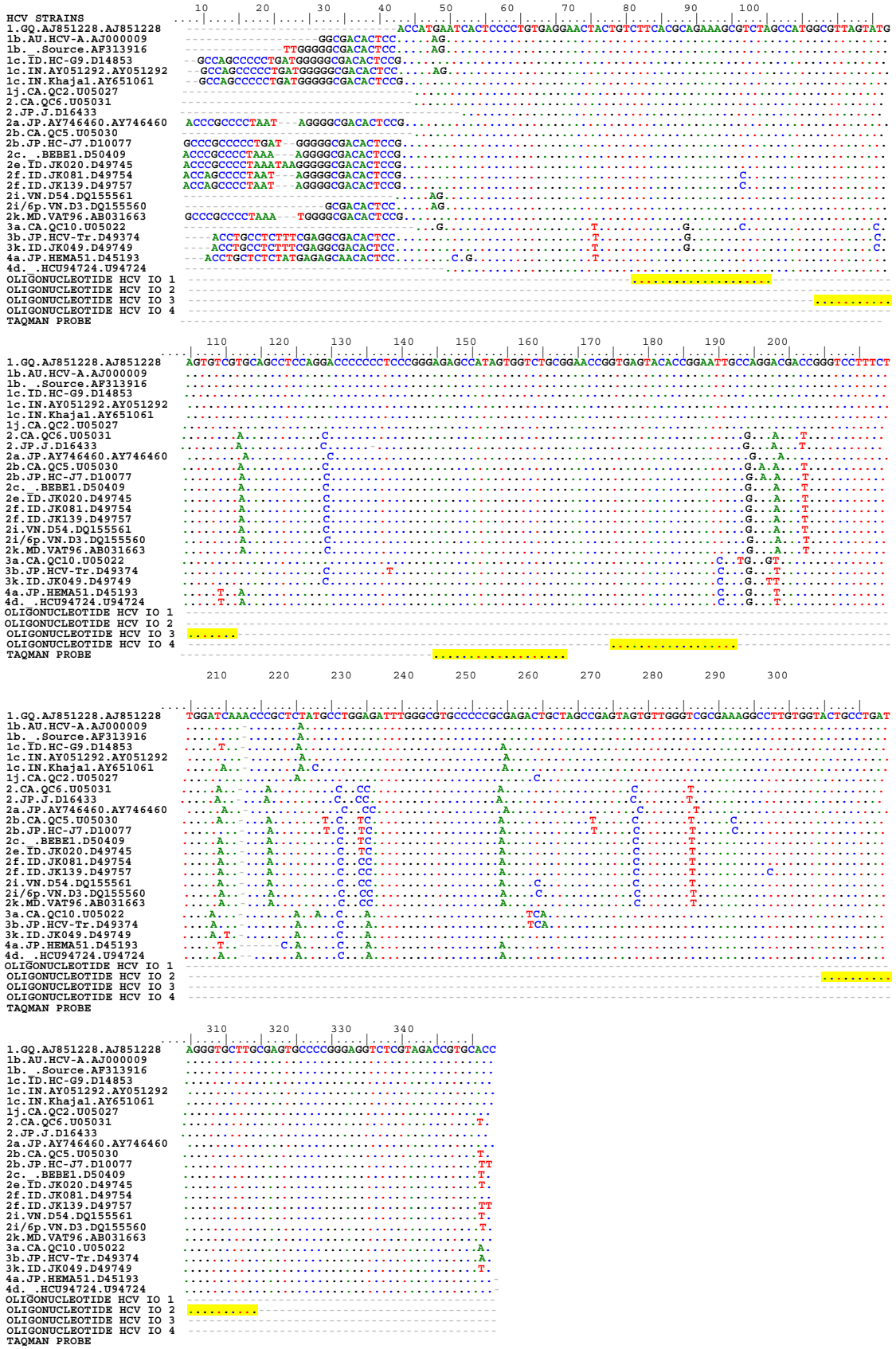

Fig. 1 Design of the hepatitis C virus (HCV) nested polymerase chain reaction (PCR) in the 5′UTR region

Table 1 Baseline characteristics of the patients included in the study

Characteristic	
N	59
Gender (male)[a]	49 (83.05)
Age (years)[b]	43.2 (38.4–48.1)
AIDS defining-criteria in the past[a]	21 (35.6)
Baseline CD4+ cell count (cells/mL)[b]	404 (257–577)
Nadir CD4+ cell count (cells/mL)[b]	237 (69–349)
Undetectable HIV viral load[a]*	56 (94.9)
On HAART[a]	58 (98.3)
Estimated time since HCV viral clearance (years)[b]	9.8 (5.3–14.6)
Baseline ALT (IU/mL)[b]	22 (17–30)
Baseline AST (IU/mL)[b]	26 (19–30)
Baseline total fasting cholesterol (mg/dL)[b]	196 (171–217)
Baseline fasting triglycerides (mg/dL)[b]	144 (99–237)
Baseline platelet count (cells/mL)[b]	20.8 (15.7–24.7)
Liver cirrhosis[a]	3 (5.08 %)
IL28B CC genotype[a]	41 (69.5 %)

N Number of cases; *IQR* interquartile range; *AIDS* acquired immunodeficiency syndrome; *HIV* human immunodeficiency virus; *mL* milliliter; *HAART* highly active antiretroviral treatment; *IU/mL* international unit per milliliter; *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *IL28B* interleukin 28B

[a] Number of cases (%)

[b] Median (IQR)

*HIV viral load was measured by polymerase chain reaction (PCR; Cobas TaqMan, Roche Diagnostic Systems, Inc., Pleasanton, CA, USA), with the detection limit set at 20 IU/mL

that are able to amplify an expanded number of HCV quasispecies.

The prevalence of liver fibrosis among HCV aviremic patients without other associated factors could be high [10, 11]. However, HCV residual replication was not observed; thus, the origin of the liver damage could not be established [10, 11]. In another study, HCV-RNA persistence in liver tissue was identified in a small number of HCV aviremic subjects with normal alanine aminotransferase (ALT) levels [5]. In one patient, the histological finding could be consistent with a chronic HCV infection. In our study, intrahepatic HCV-RNA was evaluated in two patients with significant liver fibrosis, without evidence of HCV viral persistence, suggesting that, despite being HCV aviremic, a subject could develop advanced liver fibrosis; residual HCV is an unlikely cause of this fibrosis.

The main limitation of our study is that not all patients who reached spontaneous HCV clearance identified in our cohort were enrolled in the study, which could limit the conclusions derived from the investigation. However, in interventional studies, it is not unusual for patients to refuse to participate.

Our study finds that HCV viral persistence is not a frequent situation in HIV-infected patients who have spontaneously resolved an HCV infection. Thus, at least in this subset of patients, the lack of serum HCV-RNA should continue to be addressed as the standard of healing.

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