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Negative impact of HIV infection on broad-spectrum anti-HCV neutralizing antibody titers in HCV-infected patients with advanced HCV-related cirrhosis

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ABSTRACT

Objectives: The current study aimed to assess the impact of HIV on the production of anti-HCV antibodies in HCV-infected individuals with advanced HCV-related cirrhosis before and 36 weeks after the sustained virological response (SVR) induced by direct-acting antivirals (DAAs) therapy.
Methods: Prospective study on 62 patients (50 HIV/HCV-coinfected and 12 HCV-monoinfected). Plasma anti-E2 and HCV-nAbs were determined respectively by ELISA and microneutralization assays.
Results: At baseline, the HCV-group had higher anti-E2 levels against Gt1a ($p = 0.012$), Gt1b ($p = 0.023$), and Gt4a ($p = 0.005$) than the HIV/HCV-group. After SVR, anti-E2 titers against Gt1a ($p < 0.001$), Gt1b ($p = 0.001$), and Gt4a ($p = 0.042$) were also higher in the HCV-group than HIV/HCV-group. At 36 weeks post-SVR, plasma anti-E2 titers decreased between 1.3 and 1.9-fold in the HIV/HCV-group ($p < 0.001$) and between 1.5 and 1.8-fold in the HCV-group ($p \leq 0.001$). At baseline, the HCV-group had higher titers of HCV-nAbs against Gt1a ($p = 0.022$), Gt1b ($p = 0.002$), Gt2a ($p < 0.001$), and Gt4a ($p < 0.001$) than the HIV/HCV-group. After SVR, HCV-nAbs titers against Gt1a ($p = 0.014$), Gt1b ($p < 0.001$), Gt2a ($p = 0.002$), and Gt4a ($p = 0.004$) were also higher in the HCV-group. At 36 weeks post-SVR, HCV-nAbs decreased between 2.6 and 4.1-fold in the HIV/HCV-group ($p < 0.001$) and between 1.9 and 4.0-fold in the HCV-group ($p \leq 0.001$).
Conclusions: HIV/HCV-coinfected patients produced lower levels of broad-spectrum anti-HCV antibodies than HCV-monoinfected patients.

List of abbreviations: AUC, area under the curve cART combined antiretroviral therapy; CTP, Child-Turcotte-Pugh; DAAs, direct-acting antivirals; DMEM, Dulbecco's Modified Eagle Medium; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; HCV, hepatitis C virus; HCVcc, cell-culture infectious HCV; HCV-nAbs, neutralizing antibodies against HCV; HIV, human immunodeficiency virus; HVPG, hepatic venous pressure gradient; IFN, interferon; IQR, interquartile range; LSM, liver stiffness measurement; MSM, men who have sex with men; PWID, people who inject drugs; SPSS, statistical package for the social sciences; SVR, sustained virologic response.

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1. Background

Hepatitis C virus (HCV) chronically infects about 58 million people worldwide, resulting in approximately 290,000 deaths every year [1]. Chronic hepatitis C promotes hepatic cirrhosis, end-stage liver disease, and hepatocarcinoma [2–6]. Direct-acting antivirals (DAAs) reach high rates (>95%) of sustained virologic response (SVR), even in patients with advanced HCV-related cirrhosis [7–9]. DAAs have revolutionized HCV treatment, and they have also been proposed as a tool for controlling the HCV epidemic [10,11]. However, several concerns still need to be addressed [12–14]. Around 80% of HCV-infected people are unaware of their condition, and 5% of patients who know they are infected have not received HCV treatment yet [15]. In developed countries, most people at risk are difficult to diagnose and link to care by national health systems, such as people who inject drugs (PWID), men who have sex with men (MSM), sex workers, prisoners, migrants, etc. [16]. Another challenge is the risk of HCV reinfection after DAAs therapy [17–21]. This is particularly relevant for patients coinfected with the human immunodeficiency virus (HIV) due to their immunosuppressed conditions [18,22]. The emergence of drug-resistant variants and the subsequent DAA treatment failure [23], the lack of protective immunity generated during the chronic infection [24], and severe liver damage even after achieving SVR with DAA treatment [13,25] are other reasons encouraging the development of a prophylactic vaccine.

Coinfection with human immunodeficiency virus (HIV) and HCV is a significant global public health due to shared transmission routes [26], particularly in risk groups such as PWID and MSM [27–29]. HIV-infected patients have an impaired immune system, which increases the risk of HCV infection [30], and cirrhosis follows an accelerated course in the coinfected population [31]. Moreover, a recent real-life study showed a lower efficacy of DAAs in HIV-coinfected patients than in HCV-monoinfected individuals [32]. Despite this, HCV eradication may be achievable among HIV-infected people [33].

The glycoprotein E2 is the main target of neutralizing antibodies against HCV (HCV-nAbs) [34]. There is accumulating evidence that HCV-nAbs may protect from and clear the acute HCV infection. Passive immunization with HCV-nAbs from a chronically infected individual induced protection in chimpanzees against an HCV homologous genotype but not against heterologous genotypes [35]. The infusion of HCV-nAbs prevented infection by both homologous and heterologous HCV genotypes in human liver chimeric mice [36,37]. High-titers of HCV-nAbs are related to spontaneous HCV clearance in patients with acute hepatitis C [38–40]. Remarkably, a patient with chronic hepatitis C resolved the infection after spontaneously developing HCV-nAbs [41]. Moreover, the structural characterization of the E2 HCV glycoprotein led to identifying different domains and conserved antigenic regions in this protein among different HCV genotypes [42–44]. Together with the identification and characterization of potent human HCV-nAbs, these findings gave a renewed impulse to the studies aimed to induce HCV-nAbs that prevent infection in high-risk populations.

Some reports suggest an association between the breadth of humoral immune responses and the spontaneous HCV clearance [38,39,45]. Anti-HCV antibodies have been reported to decline rapidly in HCV-monoinfected [46–48] and HIV/HCV-coinfected [49–51] patients with acute HCV infection. Nevertheless, little is known about HCV antibody levels and dynamics in chronic HIV/HCV-coinfected patients [52,53]. Furthermore, the rate at which anti-HCV antibodies are lost after DAAs therapy among HIV/HCV-coinfected patients with advanced cirrhosis remains unknown. Studies addressing this issue will inform the degree of protection of cured patients and the risk of reinfections [52,53].

This study aims to assess the impact of HIV on the production of anti-HCV antibodies in HCV-infected individuals with advanced HCV-related cirrhosis before and after DAAs therapy-induced SVR.

2. Patients and methods

2.1. Design and patients

We performed a prospective study on 62 patients with advanced HCV-related cirrhosis (50 HIV/HCV-coinfected and 12 HCV-monoinfected patients) who started HCV therapy with all-oral DAAs. Patients were recruited from the prospective ESCORIAL cohort in Madrid (Spain) between January 2015 and June 2016 (see Appendix).

Inclusion criteria were as follows: 1) chronic HCV infection with detectable serum HCV RNA levels; 2) severe cirrhosis, which included one or more clinical events related to advanced cirrhosis (liver stiffness measurement (LSM) $\geq$ 25 kPa, hepatic venous pressure gradient (HVPG) $\geq$ 10 mmHg, or Child-Turcotte-Pugh (CTP) $\geq$ 7) or history of hepatic decompensation (ascites, bleeding esophageal varices, or hepatic encephalopathy); 3) beginning all-oral DAAs therapy and achieving SVR (undetectable plasma HCV load at 12 weeks after completion of DAAs therapy); and 4) frozen plasma samples available at baseline (the time patient started anti-HCV therapy with all-oral DAAs) and the end of follow-up (week 36 after SVR).

Clinical data were collected prospectively by the ESCORIAL cohort using an online form, and the information was monitored for data verification. Blood samples were drawn and sent the same day to HIV HGM BioBank (http://hivhgmbiobank.com/?lang=en), processed, and plasma samples were stored at − 80 °C.

2.2. Laboratory assays

2.2.1. Cell culture

Human hepatoma-derived Huh7.5 cells were obtained from Apath LLC (Brooklyn, NY, USA), and Huh7.5.1 clone 2 was kindly provided by Dr. Francis V. Chisari (The Scripps Research Institute, La Jolla, CA, USA). Both cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM; Lonza, Basel, Switzerland), supplemented with 10% fetal bovine serum (FBS; Biological Industries, Beit Haemek, Israel), 4 mM L-glutamine (Lonza), and antibiotics (100 U/mL penicillin, 100 U/mL streptomycin; Lonza) at 37 °C, 5% CO_2. The cells were split every 2–3 days.

2.2.2. Expression and purification of recombinant HCV-E2 glycoproteins

The DNA sequences encoding the ectodomain of the E2 glycoprotein (residues 384–661; $E2_{661}$) of Gt1a (H77; GenBank accession no. EU363761), 1b (J4; accession no: FJ230881), 2a (JFH1; accession no: AB047639), 3a (S52; accession no: EU204645), and 4a (ED43; accession no: EU363760) with the addition of a six-histidine tag (His tag) at the 5′ end were inserted into a baculovirus transfer vector pAcGP67A (Pharmingen, San Diego, CA, USA), and expressed and purified as described previously [54] with minor modifications (Supplementary Methods).

2.2.3. Chimeric viruses

The plasmid encoding the JFH1 genome (Gt2a) was obtained from Apath LLC [55]. The plasmids encoding JFH1-based chimeric viruses containing the Core-NS2 region of Gt1a (H77/JFH1) [56], Gt1b (J4/JFH1) [57], Gt3a (S52/JFH1) [58], and Gt4a (ED43/JFH1) [56] were kindly provided by Jens Bukh (Copenhagen University Hospital, Copenhagen, Denmark). Cell-culture infectious HCVs (HCV_{cc}) were produced from plasmid-transcribed RNAs as reported in Supplementary Methods.

2.2.4. Antibody titration assays

Enzyme-linked immunosorbent assay (ELISA) titration and HCV neutralization assays were carried out as previously described [53] with slight modifications (Supplementary Methods). Patient plasma was analyzed at baseline and 36 weeks after SVR.

Patient plasma was analyzed in an ELISA using four recombinant HCV-E2 glycoproteins of Gt1a (H77), Gt1b (J4), Gt3a (S52), and Gt4a

(ED43) as antigens. These genotypes matched the HCV genotypes infecting the patients of the study.

HCV neutralization assay was performed against Gt2a (JFH1) and four JFH1-derived chimeric HCV viruses expressing E1/E2 proteins of Gt1a (H77), Gt1b (J4), Gt3a (S52), and Gt4a (ED43). These chimeric HCV viruses contain the 3′ and 5′ ends and the NS3-NS5B region of JFH1 (essential for replication and production of viral particles), and the core-NS2 region from the selected genotypes [59–61]. Although no patients were infected with Gt2a, we included the HCV Gt2a (JFH1) in this study because the non-structural part of the chimeric viruses was derived from this virus.

Non-linear regression one-phase decay curves were made using GraphPad Prism v9.0 (GraphPad Software, Inc., San Diego, CA, USA), and the area under the curve (AUC) was calculated with the same program. The percentage of neutralization was calculated as [1-(foci in the presence of plasma samples/foci in the presence of plasma control)] x 100%. Plasma control was a pool of plasma samples from individuals negative for anti-HCV antibodies determined by Murex anti-HCV v4.0 (DiaSorin Diagnostics; Dartford, UK).

2.3. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 25.0 software (SPSS INC, Armonk, NY, USA). Qualitative variables were presented as frequency and percentage, while quantitative variables were expressed as median values (interquartile range = IQR). The Wilcoxon test was used to compare data between two dependent groups, and the Mann-Whitney test to compare two independent groups. A p-value < 0.05 was considered statistically significant. Correlations were examined using Pearson's rank test, which gives us the correlation coefficient (r) that was interpreted as strong (r = 0.7–1), moderate (r = 0.5–0.7), or weak (r = 0.3–0.5), after considering significant values ($p \leq 0.05$).

3. Results

3.1. Patient characteristics

Table 1 shows the baseline characteristics of 50 HIV/HCV-coinfected and 12 HCV-monoinfected patients. HIV/HCV-coinfected patients were slightly younger, with a higher proportion of men, and less exposed to prior interferon (IFN)α therapy than HCV-monoinfected patients ($p < 0.05$). All HIV/HCV-coinfected were on combined antiretroviral therapy (cART) with undetectable HIV viral load (<50 copies/mL). Twenty-three HIV/HCV-coinfected patients were coinfected with HCV Gt1a, nine with Gt1b, seven with Gt3, and 10 with Gt4. Nine HCV-monoinfected patients were infected with HCV Gt1a, one with Gt1b, and two with Gt3. Detailed information on HCV genotypes and antiviral treatments for each patient are shown in **Suppl.** Table 1**.**

Table 1
Summary of baseline epidemiological and clinical characteristics of patients with advanced HCV-related cirrhosis.

Characteristics	HCV	HIV/HCV	p-value
No.	12	50	–
Epidemiological data			
Age (years), median [IQR]	59.9 [54.3-71.3]	52.2 [48.8-54.1]	**0.002**
Gender (male), *n* (%)	8 (66.7)	39 (78)	0.411
Current smoker, *n* (%)	8 (66.7)	34 (68)	0.999
Alcohol drinker (>50 g/day), *n* (%)	4 (33.3)	29 (58)	0.121
Intravenous drug users, *n* (%)	0 (0)	38 (76)	**< 0.001**
Previous IFNα therapy, *n* (%)	10 (83.3)	23 (46)	**0.021**
Liver disease markers			
LSM (kPa), median [IQR]	29.9 [27-66.4]	32.8 [21.9-39.5]	0.293
CTP, median [IQR]	5[5-7]	5[5-5]	0.107
HVPG (mmHg), median [IQR]	17.8 [13.5-20.5]	15[11-17]	0.123
Decompensation, *n* (%)	7 (58.3)	20 (40)	0.251
HCV markers			
Genotype 1, *n* (%)	10 (83.3)	32 (64)	0.348
Genotype 3, *n* (%)	2 (16.7)	7 (14)	0.999
Genotype 4, *n* (%)	0 (0)	10 (20)	0.191
Genotype unknown, *n* (%)	0 (0)	1 (2)	0.999
Log_{10} HCV-RNA (IU/mL), median [IQR]	6.2 [5.6–6.4]	6.2 [5.7–6.7]	0.569
HCV-RNA ≥ 850.000 IU/mL, *n* (%)	8 (66.7)	33 (66)	0.965
HIV markers			
Prior AIDS, *n* (%)	–	18 (36)	–
Nadir $CD4^+$ ($cells/mm^3$), median [IQR]	–	114.7 [70-182]	–
Nadir $CD4^+$ < 200 $cells/mm^3$, *n* (%)	–	35 (76.1)	–
Baseline $CD4^+/mm^3$, median [IQR]	–	439 [234-721]	–
$CD4^+$ < 500 $cells/mm^3$, *n* (%)	–	30 (60)	–
Antiretroviral therapy			
NRTI + NNRTI, *n* (%)	–	7 (14)	–
NRTI + II, *n* (%)	–	24 (48)	–
NRTI + IP, *n* (%)	–	7 (14)	–
IP + II + NNRTI/MVC, *n* (%)	–	4 (8)	–
Other, *n* (%)	–	8 (16)	–

Statistics: Values were expressed as absolute number (percentage) and median (interquartile range). *P*-values were calculated by Chi-square, Fisher's exact test, and Mann-Whitney tests, as required.
Abbreviations: AIDS = acquired immunodeficiency syndrome; CTP = Child-Pugh-Turcotte; HCV = hepatitis C virus; HCV-RNA = HCV plasma viral load; HIV = human immunodeficiency virus; HVPG = hepatic venous pressure gradient; IFNα = interferon-alpha; II = integrase inhibitor; IP = protease inhibitor; IQR = interquartile range; LSM = liver stiffness measure; MVC = maraviroc; NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor

3.2. Plasma anti-E2 antibodies against purified E2 proteins

We titrated antibodies in plasma samples against four purified E2 proteins corresponding to the four HCV genotypes found in this study. All patients had detectable antibodies against recombinant proteins of Gt1a (Suppl. Fig. 1 & 2), Gt1b (Suppl. Fig. 3 & 4), Gt3a (Suppl. Figures 5 **& 6**), and Gt4a (Suppl. Figures 7 **& 8**) both at baseline and 36 weeks after SVR.

Plasma anti-E2 antibody titers in HIV/HCV-coinfected and HCV-monoinfected patients showed AUC values following the decreasing order 1a > 1b > 4a > 3a, both at baseline (Suppl. Figures 9A **& C**) and 36 weeks after SVR (Suppl. Figures 9B **& 9D**).

3.2.1. Anti-E2 antibody levels are lower in HIV/HCV-coinfected patients

Comparisons of plasma anti-E2 antibody titers by patient groups (HIV/HCV vs. HCV) and study times (baseline vs. 36 weeks after SVR) are shown in Fig. 1 and Suppl. Table 2. At baseline, HCV-monoinfected patients had higher anti-E2 antibody levels against Gt1a (2.4-fold; $p = 0.012$), Gt1b (1.5-fold; $p = 0.023$), and Gt4a (1.9-fold; $p = 0.005$) than HIV/HCV-coinfected patients. At 36 weeks after SVR, anti-E2 antibody titers of Gt1a (2.8-fold; $p < 0.001$), Gt1b (1.8-fold; $p = 0.001$), and Gt4a (1.6-fold; $p = 0.042$) were also significantly higher in HCV-monoinfected than HIV/HCV-coinfected patients.

3.2.2. Anti-E2 antibody levels decrease after SVR

During the follow-up, plasma anti-E2 antibody titers against all recombinant HCV E2 glycoproteins decreased significantly between 1.3 and 1.9-fold in HIV/HCV-coinfected patients ($p < 0.001$) and between 1.5 and 1.8-fold in HCV-monoinfected patients ($p \leq 0.001$) (Fig. 1 and Suppl. Table 2). Besides, we compared the variation in plasma anti-E2 antibodies titers between patient groups (HIV/HCV vs. HCV) during the follow-up. No significant differences between groups were found (*p*

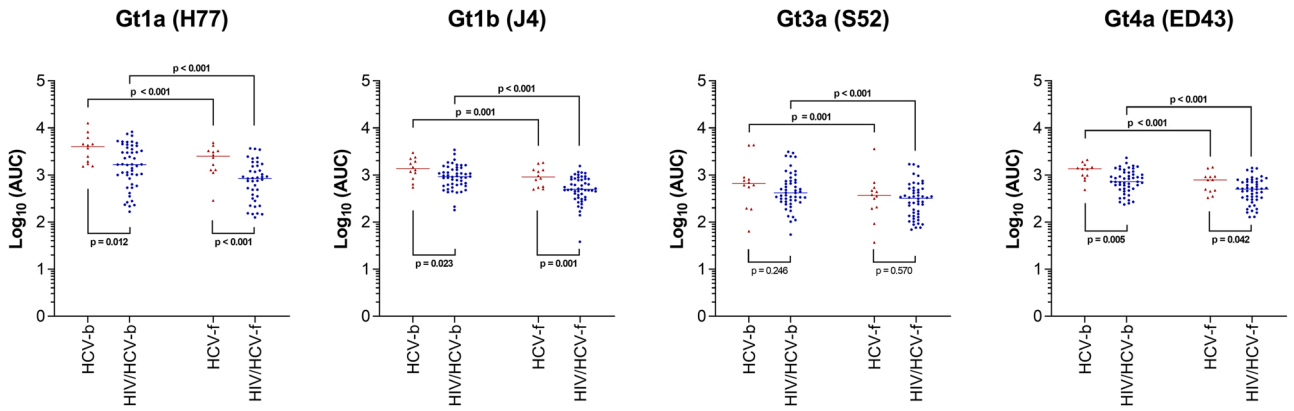

Fig. 1. Plasma antibody levels against E2 glycoproteins from different HCV genotypes in HIV/HCV-coinfected (blue circles) and HCV-monoinfected (red triangles) patients quantified by ELISA. **Statistics**: The median is represented by a horizontal line. The Wilcoxon test was used to analyze the repeated measurements within each group. The Mann-Whitney test was used to compare independent groups. The significance level was set as $p \leq 0.05$. **Abbreviations**: AUC = area under the curve (arbitrary units); Gt = HCV genotype; HCV = hepatitis C virus; HCV-b = HCV-monoinfected patients at baseline; HCV-f = HCV-monoinfected patients 36 weeks after sustained virological response; HIV = human immunodeficiency virus; HIV/HCV-b = HIV/HCV-coinfected patients at baseline; HIV/HCV-f = HIV/HCV-coinfected patients 36 weeks after sustained virological response.

> 0.05; Suppl. Table 3).

3.3. HCV-nAbs against chimeric HCV viruses

We evaluated the plasma HCV-nAbs levels against four chimeric HCV viruses corresponding to the four HCV genotypes found in this study. Plasma samples from all patients neutralized Gt1a, Gt1b, Gt2a, and Gt4a efficiently, while the HCV-nAbs levels against chimeric HCV Gt3a were low or undetectable in most patients (Suppl. Figures 10–13).

For HIV/HCV-coinfected patients, HCV-nAbs titers showed the following decreasing order: 2a > 1b > 4a > 1a > 3a, at baseline (Suppl. Figs. 14A) and 2a > 1b > 1a > 4a > 3a at 36 weeks after SVR (Suppl. Figure 14B). No HCV-nAbs were detected against Gt3a in five plasma samples at baseline and in 27 plasma samples at 36 weeks after SVR in HIV/HCV coinfected patients. HCV-nAbs were also not detected against Gt4a in four plasma samples at 36 weeks after SVR. For HCV-monoinfected patients, HCV-nAbs titers showed the following decreasing order: 2a > 4a > 1b > 1a > 3a at baseline (Suppl. Figs. 14C) and 2a > 1b > 4a > 1a > 3a and at 36 weeks after SVR (Suppl. Figure 14D). Moreover, HCV-nAbs were not detected against Gt3a in one sample at baseline and in six plasma samples at 36 weeks after SVR in HCV-monoinfected patients.

3.3.1. HCV-nAbs titers are lower in HIV/HCV-coinfected patients

At baseline, HCV-monoinfected patients had higher HCV-nAbs titers against Gt1a (4.6-fold; $p = 0.022$), Gt1b (2.4-fold; $p = 0.002$), Gt2a (4.3-fold; $p < 0.001$), and Gt4a (4.5-fold; $p < 0.001$) than HIV/HCV-coinfected patients (Fig. 2 and Suppl. Table 4). At 36 weeks after SVR, HCV-nAbs titers against Gt1a (3.0-fold; $p = 0.014$), Gt1b (4.2-fold; $p < 0.001$), Gt2a (4.8-fold; $p = 0.002$), and Gt4a (7.1-fold; $p = 0.004$) were also higher in HCV-monoinfected than in HIV/HCV-coinfected patients (Fig. 2 and Suppl. Table 4).

3.3.2. HCV-nAbs titers decrease after SVR

During the follow-up, plasma HCV-nAbs titers against all chimeric HCV viruses decreased significantly between 2.6 and 4.1-fold in HIV/HCV-coinfected patients ($p < 0.001$) and between 1.9- and 4.0-fold in HCV-monoinfected patients ($p \leq 0.001$) (Fig. 2 and Suppl. Table 4). However, the decrease in HCV-nAbs titers during the follow-up was significantly higher for genotype Gt1b ($p = 0.003$), Gt2a ($p = 0.002$), and Gt4a ($p < 0.001$) in the HIV/HCV group (Suppl. Table 5).

3.4. Correlation analysis between anti-E2 and HCV-nAbs titers

We found a strong positive correlation between anti-E2 antibody and HCV-nAbs titers in HIV/HCV-coinfected patients against all HCV genotypes at baseline (HIV/HCV-b) and 36 weeks after SVR (HIV/HCV-f)

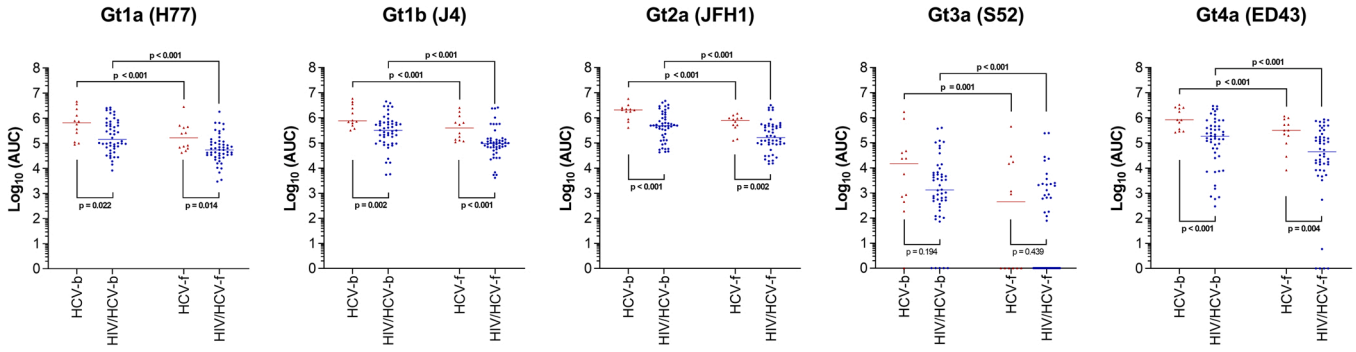

Fig. 2. Plasma titers of anti-HCV neutralizing antibodies against different chimeric HCVs expressing E1 and E2 from different genotypes in HIV/HCV-coinfected (blue circles) and HCV-monoinfected (red triangles) patients determined by microneutralization assays. **Statistics**: The median is represented by a horizontal line. The Wilcoxon test was used to analyze the repeated measurements within each group. The Mann-Whitney test was used to compare independent groups. The significance level was set as $p \leq 0.05$. **Abbreviations**: AUC = area under the curve (arbitrary units); Gt = HCV genotype; HCV = hepatitis C virus; HCV-b = HCV-monoinfected patients at baseline; HCV-f = HCV-monoinfected patients 36 weeks after sustained virological response; HIV = human immunodeficiency virus; HIV/HCV-b = HIV/HCV-coinfected patients at baseline; HIV/HCV-f = HIV/HCV-coinfected patients 36 weeks after sustained virological response.

($p < 0.05$; Fig. 3). In the HCV-group, we only found significant correlation at baseline against Gt1a ($r = 0.731$; $p = 0.007$) and Gt1b ($r = 0.600$; $p = 0.044$) and at 36 weeks after SVR against Gt1a ($r = 0.595$; $p = 0.041$) (Fig. 3), possibly due to the low number of patients in this group.

We also analyzed possible correlations between anti-E2 levels or HCV-nAbs titers and clinical variables such as age, log_{10} HCV-RNA, $CD4^+$ T-cells/mm^3, CD4/CD8 ratio, or liver markers, but no significant associations were observed (*data not shown*). However, it should not be ruled out that this lack of correlation could be due to the low sample size.

4. Discussion

The effect of HIV on the humoral response in people with advanced HCV-related cirrhosis is poorly understood. To our knowledge, this is the first report showing the titers and evolution of neutralizing HCV antibodies in HIV/HCV-coinfected patients who cleared HCV infection by DAAs treatment. Our main finding was that HIV/HCV-coinfected patients had lower plasma titers of broad-spectrum anti-E2 antibodies and HCV-nAbs than HCV-monoinfected individuals, both during chronic HCV infection and after HCV clearance with DAAs treatment. Our study provides functionally relevant data, as these HCV antibodies correlate with HCV protection and HCV clearance [62].

HCV is genetically diverse, including eight genotypes and more than 90 subtypes [63], with differences at the nucleotide level of

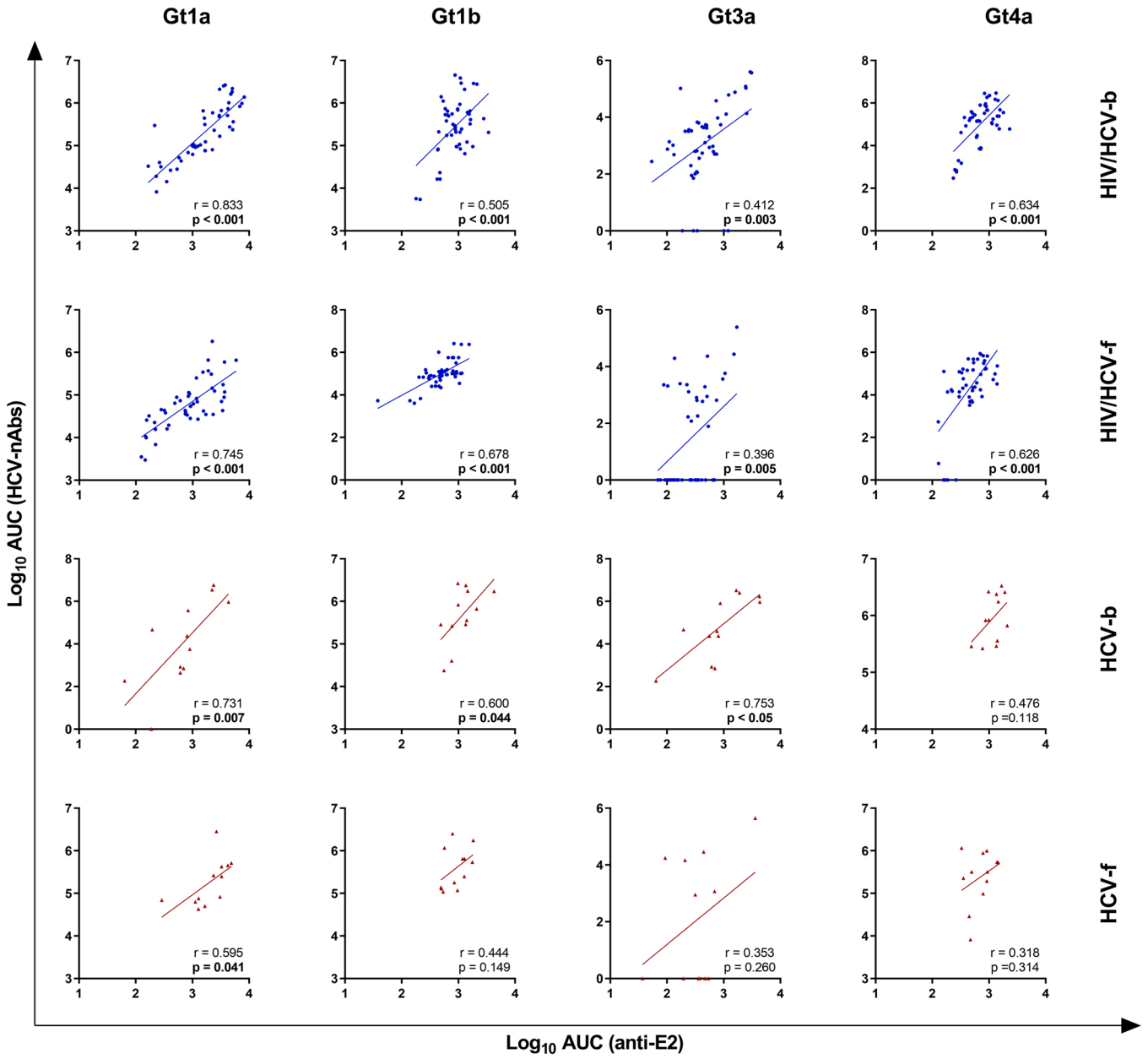

Fig. 3. Correlation between the anti-E2 antibody (anti-E2) and anti-HCV neutralizing titers (HCV-nAbs) at baseline and the end of follow-up against different HCV genotypes in HIV/HCV-coinfected (blue circles) and HCV-monoinfected (red triangles). **Statistics**: Pearson's coefficient was used to analyze the correlation between variables. The significance level was set as $p \leq 0.05$. **Abbreviations**: AUC = area under the curve (arbitrary units); Gt = HCV genotype; HCV = hepatitis C virus; HCV-b = HCV-monoinfected patients at baseline; HCV-f = HCV-monoinfected patients 36 weeks after sustained virological response; HIV = human immunodeficiency virus; HIV/HCV-b = HIV/HCV-coinfected patients at baseline; HIV/HCV-f = HIV/HCV-coinfected patients 36 weeks after sustained virological response.

approximately 30% between genotypes and 15–25% between subtypes [64]. Therefore, we analyzed neutralization titers of the plasma samples against chimeric HCV viruses based on JFH1 that express the E1 and E2 glycoproteins of five different genotypes. This study showed that most patients had antibodies that neutralized all the genotypes tested to a greater or lesser extent, as has already been seen in other studies [53,56,65]. The lower neutralization titer was against Gt3a in both patients, as also observed in other reports [53,56,65]. Moreover, patients with high anti-HCV antibody titers against a particular genotype also had high titers against different genotypes, indicating that the breadth of anti-HCV antibody responses during chronic HCV infection was independent of the infecting HCV genotype detected in the diagnostic test. Chronic hepatitis C is asymptomatic for several years after the initial infection. Therefore, HCV-infected patients are usually not detected until the liver is seriously damaged. People may become infected several times by different HCV genotypes during this period, particularly those from high-risk populations. However, just one genotype usually predominates inside each individual at a time. This may explain why we do not detect a pool of different HCV genotypes at baseline. However, successive contact with different genotypes may have boosted the production of anti-HCV cross-reactive antibodies, as has been observed in other natural infections and vaccine strategies [66,67]. On the other hand, some regions of the E2 glycoprotein, the main target of nAbs, are conserved among different HCV genotypes, which would also justify the response against other HCV genotypes that did not infect the patient [42].

HCV infection triggers an immune response producing HCV-nAbs [68]. Thus, most of our patients had high titers of anti-E2 antibodies and HCV-nAbs at baseline. However, this immune response may be affected by many factors, among which HIV infection is one of the most important. HIV infection precipitates the onset of HCV-related cirrhosis [69] and leads to impaired immune response, even in patients receiving successful antiretroviral therapy [70,71]. In our study, despite suppressive cART, HIV/HCV-coinfected patients had lower titers of anti-E2 antibodies and HCV-nAbs than the HCV-monoinfected individuals. This may be due to the memory B lymphocytes and plasma cell disorders in HIV-infected patients [72,73], an impact that is not entirely restored by the cART [73]. Besides, HIV infection impairs the immunoglobulin class switching [74,75], penalizing the production of anti-HCV antibodies with high affinity and broad-spectrum [76,77]. This effect is not exclusive of HIV/HCV coinfected patients since HIV patients coinfected with other pathogens (measles or pneumococcus) also show a decreased production of specific antibodies against these microorganisms compared to HIV-uninfected patients [73,78].

In our work, HCV clearance after DAAs therapy resulted in an evident decline in anti-E2 antibody levels and HCV-nAbs titers after 36 weeks. These results align with previous findings showing that HIV-infected patients treated during acute [51] or chronic [49] HCV infection who achieved SVR with IFN-based therapy substantially decay anti-HCV antibody levels. A previous study of our group that involved HIV/HCV-coinfected patients also showed a decline in HCV-nAbs at 24 weeks after achieving SVR with IFN-based therapy [53]. The present study extends and confirms these results and suggests that HCV clearance could result in the loss of antigenic stimulation required for a sustained antibody response. Monitoring anti-HCV antibody dynamics following SVR could help evaluate the risk of HCV reinfection in a population of HIV-infected patients. In this regard, although HCV-nAbs developed during HCV chronic infection do not lead to virus clearance, it should be stressed that they may protect from reinfection after SVR, as suggested by passive administration of anti-HCV antibody experiments [35,38,79,80]. This idea is also supported by the observation that HCV can only be cleared if a rapid and potent HCV-nAbs response occurs shortly after virus infection, while a delayed antibody response favors chronicity [39,81–83].

Overall, the lower HCV-nAbs levels in the HIV/HCV group and their decrease after DAA therapy could explain the higher HCV reinfection rates observed in this population and must be considered in developing future anti-HCV vaccines [84–86]. However, we did not evaluate the level of protection offered by the neutralizing HCV antibodies measured in this study. This is an important issue, and the answer to this question will be highly relevant for developing an antibody-based vaccine against HCV and understanding viral reinfections.

4.1. Limitations of this study

Firstly, our study had a small number of patients, which directly affected the power and statistical significance of the results. However, the repeated measures design considerably improve the power of the analysis. Secondly, all participants had advanced HCV-related cirrhosis, which may limit the generalizability of our results. Additional studies need to be performed to assess the impact of HIV infection in non-cirrhotic patients. Besides, the HIV/HCV group was not exactly similar to the HCV group. Thirdly, the lack of a broader library of chimeric HCV viruses from clinical strains [87] to assess the neutralization capacity against other genotypes such as Gt5, 6, or 7. Finally, the lack of information on anti-E2 memory B lymphocytes in the blood.

5. Conclusions

HIV/HCV-coinfected patients produced lower levels of broad-spectrum anti-HCV antibodies than HCV-monoinfected patients. The lower plasma titers of anti-E2 and HCV-nAbs in HIV/HCV-coinfected patients and their decline after DAA therapy should be considered in the development of future HCV vaccines.

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Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Instituto de Salud Carlos III (CEI PI 41_2014) and performed according to the ethical guidelines of the 1975 Declaration of Helsinki. All patients gave written informed consent to participate in the cohorts.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Conceptualization: SR and IM, Methodology: DSC, SR, and IM, Software: SR, Validation: IM, Formal analysis: DSC, SR, and IM, Investigation: DSC, MBY, JG, FTF, and PG, Resources: CD, VH, JB, JGG, LIS, EL, AO, and JM, Data Curation: CD, VH, JB, JGG, LIS, EL, AO, and JM, Writing – Original Draft: DSC and IM, Writing – Review & Editing: SR, Visualization: SR and IM, Supervision: SR and IM, Funding acquisition: IM, SR, JBB, and JGG, All authors have read and approved the final manuscript.

Conflict of interest statement

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding authors upon reasonable request.

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Authors' information

Not applicable.

Appendix A

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- **Fundación SEIMC/GeSIDA** (Madrid, Spain): Marta de Miguel, María Yllescas and Herminia Esteban.

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.113024.

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