Sustained virological response after treatment with direct-acting antivirals can help immune reconstitution in HIV-HCV coinfected patients even in case of persistent HIV low-level viremia

Monica Basso1 | Daniela Zago1 | Irene Pozzetto1 | Giuliana Battagin2 | Antonio Carlotto3 | Maria Cristina Rossi4 | Sandro Panese5 | Loredana Sarmati6 | Saverio Giuseppe Parisi1

1Department of Molecular Medicine, University of Padova, Padova, Italy
2Clinical Infectious Diseases, Vicenza Hospital, Vicenza, Italy
3Infectious Diseases, Santorso Hospital, Santorso, Italy
4Infectious Diseases Unit, Treviso Hospital, Treviso, Italy
5Infectious Diseases Unit, dell’Angelo Hospital, Venezia, Italy
6Infectious Diseases Clinic, Università Tor Vergata, Rome, Italy

Correspondence
Saverio Giuseppe Parisi, Department of Molecular Medicine, University of Padova, Padova, Italy, Via Gabelli 63, 35100 Padova, Italy. Email: saverio.parisi@unipd.it

INTRODUCTION

HCV infection promotes both a specific immune response against HCV and a nonspecific immune activation, which is more severe in HIV-positive subjects: serum biomarkers of inflammation, bacterial translocation, and endothelial disfunction are significantly higher in case of HIV-HCV coinfection with respect to HIV monoinfection and a significant decrease occurred in case of HCV clearance.1,2 Once HCV is eradicated, the HIV involvement in the inflammatory process leading to liver fibrosis may disappear.3 Low-level plasma HIV viremia is a parameter that can be related to immune activation4-7: the threshold value in copies/mL is object of great debate. Lesko et al8 reported in a recently published work that common choices below which HIV viral load will be considered suppressed are 20, 50, 200, or 400 copies/mL and even higher plasma HIV-RNA levels were reported.4,9 The clinical implications of low-level viremia are still not fully clear because of the differences in populations studied and in study designs but virological failure was associated to the detection of plasma HIV-RNA values between 201 and 500 copies/mL.9-11 Now, direct-acting antiviral (DAA) therapy allows HCV eradication in over 95% of HIV-HCV coinfected subjects after a short treatment period. Hence, the treatment of HCV infection can be considered a simple clinical model of inflammatory status regression.2

The aim of this retrospective longitudinal study was to evaluate the influence of HCV-RNA clearance at SVR12 (persistently negative HCV-RNA at 12 weeks after completion of therapy) on CD4+ and CD8+ cell count and percentage and HIV virologic control.

METHODS

The study population included HIV-1 infected adults with chronic HCV infection who were treated with DAA and achieved SVR12. Inclusion criteria were the availability of three plasma HIV-RNA determination by year at least in the 2 years before anti-HCV treatment started, a successful virologic control of HIV (defined as no plasma HIV-RNA value >200 copies/mL in the 2 years before anti-HCV treatment started), and plasma HIV viremia tested within a week from SVR 12. Patients who responded to the inclusion criteria were further classified as undetectable patients (Up) when plasma HIV-RNA was
undetectable in the 2 years before the beginning of DAA treatment and as low-level viremia patients (LLVp) otherwise. A sub-analysis based on a lower viremia threshold (very low-level viremia, <40 copies/mL) was performed in LLVp. Study times: 24-month period before the start of anti-HCV treatment, including pre-DAA start (T0) and SVR12. The choice of ART and anti-HCV regimens was up to the discretion of the treating physician. CD4+ and CD8+ cell count and percentage were evaluated by flow-cytometry analysis; HIV-RNA and HCV-RNA viral loads were tested with a commercial quantitative PCR method. This study is a retrospective observational study and was approved by the local Ethic Committee: patients gave their informed consent and all data were anonymized prior to research use. Chi-squared test, Fisher’s exact test, Mann-Whitney test, and Wilcoxon test were used as appropriate. Continuous variables were described as median and interquartile range (IQR) and categorical variables as absolute numbers and percentage. Statistical analysis was performed using MedCalc Statistical Software version 19.1 (MedCalc Software by Ostend, Belgium). A P value <.05 was considered statistically significant.

3 | RESULTS

Overall, 198 patients achieved SVR12. 104 patients complied with all the inclusion criteria, and 94 were excluded (Figure S1). All the patients were Caucasian and HBsAg negative. Overall, median CD4+ cell count was 621 cells/mm³ (IQR 443-846 cells/mm³) and median nadir value of 198 cells/mm³ (IQR 87-301 cells/mm³): 59 (56.7%) were LLVp and 45 were Up (43.3%). The main viroimmunological characteristics and prescribed ART were comparable in the two groups of patients (Table 1); DAA regimen is described in Table S1. The interval between the last plasma HIV-RNA testing and the start of anti-HCV therapy was 4 weeks (median value, IQR 2-5 weeks).

Patients with undetectable plasma HIV viremia at the anti-HCV treatment start and in the 2 years before (undetectable patients, Up) were 45 while patients who experienced plasma HIV viremia <200 copies/mL in the same study points were 59.

At SVR 12, 35 (77.8%) out of the 45 Up had undetectable plasma HIV viremia, 9 (20%) had detectable plasma HIV viremia <200 copies/mL, and 1 (2.2%) patient had plasma HIV viremia >200 copies/mL; 30 (50.8%) out of the 59 LLVp had undetectable plasma HIV viremia while 29 (49.2%) had detectable plasma HIV viremia <200 copies/mL.

Of the 59 LLVp, 39 (66.1%) had all plasma HIV-RNA values <40 copies/mL before anti-HCV treatment start: 23 (58.9%) of them had undetectable HIV viremia at SVR12, a frequency higher than that found in the 20 LLVp patients with at least one plasma HIV-RNA value >40 copies before anti-HCV treatment start: 23 (58.9%) of them had undetectable HIV viremia at SVR12, a frequency higher than that found in the 20 LLVp patients with at least one plasma HIV-RNA value >40 copies before anti-HCV treatment start (35%, seven patients). HCV clearance is not associated with any modification in median CD4+ or CD8 absolute cell count and percentage at SVR12 in Up, while LLVp who had HIV-RNA undetectable at SVR12 showed a significant increase in median CD4+ cell count and percentage (658, IQR 398-829 CD4+ cell count/mm³ vs 728, IQR 487-976 CD4+ cell

| TABLE 1 | Main characteristics of the 104 HIV-HCV patients included in the study according to plasma HIV viremia control (Up: patients with undetectable plasma HIV viremia patients with plasma HIV-RNA undetectable in the 2 years before beginning the DAA treatment; LLVp: patients with low-level HIV viremia in the 2 years before beginning the DAA treatment) |
| --- | --- |
|  | Up (45 patients) | LLVp (59 patients) |
| Males, n (%) | 32 (71.1) | 44 (74.6) |
| Age (years) | 53 (50-54) | 53 (49-55) |
| CD4+ cell count at nadir (cells/mm³) | 202 (86-296) | 197 (86-306) |
| ART modified before anti-HCV therapy, n (%) | 28 (62.2) | 29 (49.2) |
| Pretreatment with HCV-RNA (IU/mL) | 1 320 000 (430775-3 372 446) | 1 850 000 (402882-4 820 750) |
| Pretreatment with F3-F4 fibrosis, n (%) | 24 (53.3) | 29 (49.2) |
| Patients treated with InSTI at HCV treatment start, n (%) | 21 (46.7) | 19 (32.2) |
| Patients treated with InSTI + PIs at HCV treatment start, n (%) | 5 (11.1) | 3 (5.1) |
| Patients treated with NNRTIs at HCV treatment start, n (%) | 8 (17.8) | 14 (23.7) |
| Patients treated with NRTIs at HCV treatment start, n (%) | 1 (2.2) | 0 |
| Patients treated with PIs at HCV treatment start, n (%) | 11 (24.4) | 22 (37.3) |

Abbreviations: ART, antiretroviral therapy; InSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors.

*At HCV treatment start.

*Median and IQR range.
count/mm³ and 33, IQR 28.5-39 CD4+ cell percentage vs 34, IQR 27.5-40.1 CD4+ cell percentage. Interestingly, LLVP with RV at SVR12 showed an increase in median CD4+ cell count/mm³ (578, IQR 505-827 CD4+ cell count/mm³ vs 659, IQR 498-951 CD4+ cell count/mm³) and percentage, this last one significant (31, IQR 24.2-35.7 CD4+ cell percentage vs 32, IQR 24.8-38.5 CD4+ cell percentage, \( P = .01 \)). The increase was significant for median CD4+ cell count and percentage when the analysis was made including only LLVP who had HIV-RNA <40 copies/mL in SVR12 (588 CD4+ cell/mm³, IQR 560-776 CD4+ cells/mm³ vs 689 CD4+ cells/mm³, IQR 548-980 CD4+ cells/mm³, \( P = .02 \) and 31%, IQR 25-36% vs 31%, IQR 27-40%, \( P = .02 \)). A complete description of CD4+ and CD8+ cell counts and percentages are reported in Table S2. The proportion of subjects who achieved HIV-RNA undetectability was comparable in those who modified ART and in those who did not both in Up (82.1% and 70.6%, respectively) and in LLVP (51.7% and 50%, respectively).

### 4 | DISCUSSION

The present study included two groups of HIV-HCV patients comparable for all the variables tested but with stable RV or stable HIV-RNA undetectability. We observed a favorable and fast effect of HCV-RNA clearance on the immunovirologic asset, and we supposed that the underlying mechanism in immunovircation status decreases. The first main result observed was the increase of CD4+ cell count and percentage (significant for this parameter) in LLVs with detectable HIV viremia at SVR12. The difference was significant for both CD4+ number and percentage in the analysis restricted to patients with plasma HIV-RNA always <40 copies/mL. These results suggested that HCV clearance seemed to have a favorable impact on CD4 recovery regardless of the persistence of low-level HIV viremia and with more significant effects in case of lower HIV-RNA loads. Many markers of immune recovery were studied and not all of them were evaluated in clinical practice: absolute CD4 positive cell count is the parameter included in almost all published definitions of immunological responder and that is why we focused on it and on CD4+ cell percentage.

Our results are in accordance with those published by Doyle et al.\(^1\) on 23 patients, and with no data on CD4 cell count percentage; the reasons for these data may be the evidence that patients cured for HCV and with low-level HIV viremia (with the cut-off of 40 copies/mL and 50 copies/mL, respectively) had monocyte activation comparable to that of subjects with HIV monoinfection and normal ranges of pro-inflammatory and regulatory cytokines.\(^14,15\)

The lower levels of T-cell activation described in patients with undetectable plasma HIV viremia with respect to those with detectable plasma HIV-RNA\(^16\) could be the basis for the second main result of our study; about half the subjects with RV achieved HIV-RNA undetectability at SVR12. We could report these data because our study design distinguished patients with detectable plasma HIV viremia (even at low copy number) and those with a negative result, differently from previously published works, including HIV-HCV patients successfully treated with DAA, that define HIV suppression as having a plasma HIV-RNA lower than 50 copies/mL.\(^17,18\) The percentage of subjects who achieved complete HIV viremia suppression was higher than that observed in a previous study including a lower number of patients.\(^19\) The choice of SVR12 in the present study instead of a 24-week interval from anti-HCV treatment start may justify the different result and reinforce our hypothesis even though we cannot exclude that some patients could have a detectable but not quantifiable HIV-RNA if retested. However, a higher adherence to ART during DAA treatment should be taken into account as well.\(^20\) We are aware that only slight differences in viroimmunological parameters were reported but, on the other hand, the patients included were immunocompetent and with an ongoing antiretroviral treatment, even if with a different level of virological suppression.

The current study has some limitations and strengths: the former are the retrospective study design and the numerosity of the four groups of patients, while the latter are the availability of plasma HIV-RNA data obtained in the 2 years before the start of the anti-HCV treatment to classify the HIV virologic control of the patients and the clinical practice approach.

In conclusion, we observed a positive influence of HCV cure in patients with low-level plasma HIV viremia before anti-HCV treatment start, possibly because of a reduction of immune activation: this is a preliminary study and these results should be confirmed after a longer follow-up.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest for this article.

### AUTHOR CONTRIBUTIONS

Conceptualization: Saverio Giuseppe Parisi

Formal analysis: Monica Basso

Investigation: Giuliana Battagin, Antonio Carlotto, Maria Cristina Rossi, Sandro Panese, Loredana Sarmati

Project Administration: Saverio Giuseppe Parisi

Resources: Saverio Giuseppe Parisi, Monica Basso

Supervision: Saverio Giuseppe Parisi

Visualization: Loredana Sarmati, Irene Pozzetto

Writing - Original Draft Preparation: Monica Basso, Daniela Zago, Irene Pozzetto

Writing - Review & Editing: Saverio Giuseppe Parisi, Monica Basso

All Authors had read and approved the final version of the manuscript.

Saverio Giuseppe Parisi had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.
TRANSPARENCY STATEMENT
Saverio Giuseppe Parisi confirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT
The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

ORCID
Saverio Giuseppe Parisi https://orcid.org/0000-0002-8865-5460

REFERENCES
1. Parisi SG, Andreis S, Mengoli C, et al. Soluble CD163 and soluble CD14 plasma levels but not cellular HIV-DNA decrease during successful interferon-free anti-HCV therapy in HIV-1 co-infected patients on effective combined anti-HIV treatment. Med Microbiol Immunol. 2018;207:183-194.

2. López-Cortés LF, Trujillo-Rodríguez M, Baez-Palomo A, et al. Eradication of hepatitis C virus (HCV) reduces immune activation, microbial translocation, and the HIV DNA level in HIV/HCV-Coinfected patients. J Infect Dis. 2018;218:624-632.

3. Corma-Gómez A, Morano L, Téllez F, et al. HIV infection does not increase the risk of liver complications in hepatitis C virus-infected patient with advanced fibrosis, after sustained virological response with direct-acting antivirals. Aids. 2019;33:1167-1174.

4. Sarangi L, D’Ettorre G, Parisi SG, Andreoni M. HIV replication at low levels in HIV-1-HCV co-infected patients during simplification therapy with protease inhibitor-sparing regimens. J Med Virol. 2015;13:250-257.

5. Sarangi L, Parisi SG, Nicasiri E, et al. Cellular HIV-1 DNA quantitation in patients during simplification therapy with protease inhibitor-sparing regimens. J Med Virol. 2007;79:880-886.

6. Nicasiri E, Palmisano L, Sarangi L, et al. HIV-1 residual viremia and proviral DNA in patients with suppressed plasma viral load (<400 HIV-RNA cp/ml) during different antiretroviral regimens. Curr HIV Res. 2008;6:261-266.

7. Elvstam O, Marrone G, Medstrand P, et al. All-cause mortality and serious non-AIDS events in adults with low-level HIV viremia during combination antiretroviral therapy: results from a Swedish Nationwide observational study. Clin Infect Dis. 2020;ciaa413. https://doi.org/10.1093/cid/ciaa413

8. Lesko CR, Chander G, Moore RD, Lau B. Variation in estimated viral suppression associated with the definition of viral suppression used. Aids. 2020;34:1519-1526.

9. Fleming J, Mathews WC, Rutstein RM, et al. Low-level viremia and virologic failure in persons with HIV infection treated with antiretroviral therapy. Aids. 2019;33:2005-2012.

10. Antiretroviral Therapy Cohort Collaboration (ART-CC), Vandenhende MA, Ingle S, et al. Impact of low-level viremia on clinical and virological outcomes in treated HIV-1-infected patients. Aids. 2015;29:373-383.

11. Elvstam O, Medstrand P, Yilmaz A, Isberg PE, Gisslén M, Björkman P. Virological failure and all-cause mortality in HIV-positive adults with low-level viremia during antiretroviral treatment. PLoS One. 2017;12: e0180761.

12. Bordoni V, Brando B, Piselli P, et al. Naïve/effecter CD4 T cell ratio as a useful predictive marker of immune reconstitution in late presenter HIV patients: a multicenter study. PLoS One. 2019;14: e0225415.

13. Doyle MA, Lee T, Singer J, Crawley A, Klein M, Cooper C. Evaluation of safety and effectiveness of Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide switch followed by Ledipasvir/Sofosbuvir HCV therapy in HIV-HCV coinfection. Open Forum Infect Dis. 2019;6:ofz318.

14. Sun B, Abadjian L, Monto A, Freasier H, Pulliam L. HCV cure in HIV coinfection dampens inflammation and improves cognition through multiple mechanisms. J Infect Dis. 2020;222:394-406.

15. Badano MN, Parodi C, Aloisi N, Corti M, Elizalde de Bracco MM, Baré P. Influence of Hepatitis C virus coinfection on immune reconstitution in HIV subjects. Med Microbiol Immunol. 2019;208:747-756.

16. Li JZ, Segal FP, Bosch RJ, et al. Antiretroviral therapy reduces T-cell activation and immune exhaustion markers in human immunodeficiency virus controllers. Clin Infect Dis. 2020;70:1636-1642.

17. Huhn GD, Ramgopal M, Jain MK, et al. HIV/HCV therapy with ledipasvir/sofosbuvir after randomized switch to emtricitabine-tenofovir alafenamide-based single-tablet regimens. PLoS One. 2020;15:e0224875.

18. Milazzo L, van den Bogaart L, Sollima S, et al. Impact of HCV eradication with direct-acting antiviral agents on serum gamma globulin levels in HCV and HCV/HIV coinfected patients. Eur J Intern Med. 2020;75:50-54.

19. Parisi SG, Andreis S, Basso M, et al. Time course of cellular HIV-DNA and low-level HIV viremia in HIV-HCV co-infected patients whose HCV infection had been successfully treated with directly acting antivirals. Med Microbiol Immunol. 2017;206:419-428.

20. Guzman Ramos MI, Manzano-García M, Robustillo-Cortés MLA, Pineda JA, Morillo-Verdugo R. Effect on the adherence to concomitant medications after initiation of treatment with direct-acting antiviral agents against hepatitis C virus. Gastroenterol Hepatol. 2020;43: 418-425.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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