

Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Table 1

Baseline characteristics of HIV/HCV-coinfected patients stratified by persistently elevated liver stiffness five years after completion of successful HCV treatment.

	Persistently elevated liver stiffness	Not persistently elevated liver stiffness	<i>p</i>-value
No.	24 (61.5%)	15 (38.5%)	
Age (years)	51 (48–53)	51 (48–53)	0.562
Gender			0.036
Male	16 (66.7%)	15 (100.0%)	
Female	8 (33.3%)	0 (0.0%)	
BMI ((kg/m ²))	24.7 (22.3–26.3)	24.6 (23.3–26.3)	0.795
Smoker			0.659
Never	3 (12.5%)	2 (13.3%)	
Previous (>6 months)	5 (20.8%)	5 (33.3%)	
Current	16 (66.7%)	8 (53.3%)	
Alcohol intake (>50g/day)			0.507
Never	11 (45.8%)	8 (53.3%)	
Previous (>6 months)	11 (45.8%)	7 (46.7%)	
Current	2 (8.3%)	0 (0.0%)	
Intravenous drug user			0.976
Never	5 (20.8%)	4 (26.7%)	
Previous (>6 months)	19 (79.2%)	11 (73.3%)	
Current			
Previous HCV therapy			0.999
Yes	13 (54.2%)	8 (53.3%)	
No	11 (45.8%)	7 (46.7%)	
MASLD (n = 37)			0.999
Yes	5 (22.7%)	3 (20.0%)	
No	18 (77.3%)	12 (80.0%)	

Liver markers

LSM (kPa) baseline	27.7 (21.8–36.3)	13.5 (11.4–26.6)	0.004
LSM (kPa) 1 year after HCV treatment	22.8 (20.6–33.9)	9.0 (7.6–13.9)	<0.001
LSM (kPa) 5 years after HCV treatment	23.5 (17.0–33.9)	7.6 (6.7–9.4)	<0.001
HSI (n = 37)	31.7 (28.2–35.4)	34.3 (33.6–36.4)	0.127
FIB-4 (n = 37)	6.7 (2.7–9.7)	2.9 (2.2–4.3)	0.056

HCV markers

HCV genotype (n = 37)			0.534
1	16 (69.6%)	12 (85.7%)	
3	4 (17.4%)	1 (7.1%)	
4	3 (13.0%)	1 (7.1%)	
Log ₁₀ HCV-RNA (IU/mL)	6.0 (5.4–6.4)	6.3 (5.8–6.7)	0.113
HCV-RNA > 850.000 IU/mL			0.340
Yes	13 (54.2%)	9 (60.0%)	
No	11 (45.8%)	6 (40.0%)	

HCV therapy

pegIFN	9 (37.5%)	13 (86.7%)	0.007
DAAs	15 (62.5%)	2 (13.3%)	

HIV markers

Previous AIDS (n = 38)			0.782
Yes	0 (0.0%)	1 (7.1%)	
No	24 (100.0%)	13 (92.9%)	
CD4+ T-cells/mm ³	372.0 (245.3–518.5)	515.0 (367.0–772.5)	0.172
CD4+ T-cells < 500 cells/mm ³			0.147
Yes	18 (75.0%)	7 (46.7%)	
No	6 (25.0%)	8 (53.3%)	

HIV antiretroviral therapy

NRTI + NNRTI	9 (37.5%)	2 (13.3%)	0.102
NRTI + II	9 (37.5%)	6 (40.0%)	
NRTI + PI	1 (4.2%)	5 (33.3%)	
PI+II+NNRTI/MVC	1 (4.2%)	0 (0.0%)	

Others

4 (16.7%)

2 (13.3%)

Statistics: The values are expressed as the absolute number (percentage) and median (interquartile range). P-values were calculated by the Chi-square test and the Mann-Whitney U test. Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; BMI, body mass index; MASLD, metabolic dysfunction-associated steatotic liver disease; LSM, liver stiffness measurement (before treatment); kPa, kilopascal; HSI, hepatic steatosis index; FIB-4, fibrosis-4; pegIFN, pegylated interferon; DAAs, direct-acting antivirals; AIDS, acquired immune deficiency syndrome; NRTI, nucleoside analogue HIV reverse transcriptase inhibitor; NNRTI, non-nucleoside analogue HIV reverse transcriptase inhibitor; II, HIV integrase inhibitor; PI, HIV protease inhibitor.

1.2 Supplementary Table 2

Association of plasma immune checkpoint proteins one year after completion of successful HCV treatment with persistently elevated liver stiffness (LSM \geq 12.5 kPa) at the end of follow-up (five years after treatment) in HIV/HCV-coinfected patients.

Marker	Un-adjusted			Adjusted		
	AMR (95%CI)	<i>p</i> -value	<i>q</i> -value	aAMR (95%CI)	<i>p</i> -value	<i>q</i> -value
BTLA	1.42 (1.04–1.95)	0.034	0.094	1.49 (1.15–1.96)	0.006	0.051
CD137(4-1BB)	1.23 (0.87–1.73)	0.250	0.472	1.05 (0.71–1.55)	0.827	0.891
CD152(CTLA4)	1.19 (0.88–1.63)	0.270	0.472	1.39 (1.00–1.90)	0.051	0.143
CD27	1.04 (0.82–1.33)	0.719	0.774	1.13 (0.88–1.46)	0.337	0.473
CD28	1.12 (0.86–1.48)	0.391	0.548	1.16 (0.91–1.48)	0.233	0.362
CD80	1.71 (1.25–2.35)	0.002	0.028	1.40 (0.99–1.97)	0.067	0.157
GITR	1.16 (0.84–1.59)	0.376	0.548	1.20 (0.91–1.60)	0.207	0.361
HVEM	0.95 (0.73–1.23)	0.703	0.774	0.99 (0.78–1.27)	0.965	0.965
IDO	0.95 (0.57–1.57)	0.833	0.833	0.46 (0.22–1.00)	0.051	0.143
LAG-3	1.27 (1.05–1.55)	0.022	0.088	1.06 (0.84–1.33)	0.653	0.762
PD-1	1.45 (1.06–1.97)	0.025	0.088	1.49 (1.14–1.97)	0.007	0.051
PD-L1	1.06 (0.87–1.29)	0.559	0.712	1.14 (0.95–1.37)	0.157	0.315
PD-L2	1.29 (1.05–1.59)	0.021	0.088	1.09 (0.87–1.37)	0.461	0.587
TIM-3	1.24 (1.01–1.51)	0.047	0.109	1.28 (1.05–1.56)	0.020	0.092

Statistics: Data were calculated by Generalized Linear Models (GLM) with a gamma distribution (log-link). Multivariable models were adjusted by age, gender, HCV treatment (IFN-based therapy or DAAs), LSM at one year after treatment, and time elapsed between the two times, which were previously selected by a stepwise method (forward) (see Results Section). The *q*-values represent *p*-values corrected for multiple testing using the False Discovery Rate (FDR; Benjamini and Hochberg procedure). Statistically significant differences are shown in bold. Abbreviations: AMR, arithmetic mean ratio; aAMR, adjusted AMR; 95%CI, 95% of confidence interval; *p*, level of significance; *q*, corrected level of significance; BTLA, B and T lymphocyte attenuator; CD, cluster of differentiation; GITR, glucocorticoid-induced TNFR-related; HVEM, herpesvirus entry mediator; IDO, indoleamine 2,3-dioxygenase; LAG-3, lymphocyte activation gene-3; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; TIM-3, T-cell immunoglobulin and mucin-domain containing-3.

1.3 Supplementary Table 3

Association of plasma immune checkpoint proteins at baseline (before HCV treatment) with persistently elevated liver stiffness (LSM \geq 12.5 kPa) at the end of follow-up (five years after treatment) in HIV/HCV-coinfected patients.

Marker	Un-adjusted			Adjusted		
	AMR (95%CI)	<i>p</i> -value	<i>q</i> -value	aAMR (95%CI)	<i>p</i> -value	<i>q</i> -value
BTLA	2.19 (1.11–4.31)	0.030	0.045	1.60 (0.85–3.01)	0.154	0.154
PD-1	2.71 (1.27–5.78)	0.014	0.042	2.71 (1.27–5.78)	0.014	0.042
TIM-3	1.26 (1.01–1.58)	0.048	0.048	1.26 (1.01–1.58)	0.048	0.072

Statistics: Data were calculated by Generalized Linear Models (GLM) with a gamma distribution (log-link). Multivariable models were adjusted by age, gender, LSM at baseline, and time elapsed between the two times, which were previously selected by a stepwise method (forward) (see Results Section). The *q*-values represent *p*-values corrected for multiple testing using the False Discovery Rate (FDR; Benjamini and Hochberg procedure). Statistically significant differences are shown in bold. Abbreviations: AMR, arithmetic mean ratio; aAMR, adjusted AMR; 95%CI, 95% of confidence interval; *p*, level of significance; *q*, corrected level of significance; BTLA, B and T lymphocyte attenuator; PD-1, programmed cell death protein 1; TIM-3, T-cell immunoglobulin and mucin-domain containing-3.