Heliyon 9 (2023) e12686

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Normalization of circulating plasma levels of miRNAs in HIV-1/HCV co-infected patients following direct-acting antiviral–induced sustained virologic response

Sandra Franco [a], Josep M. Llibre [b,c], Toni Jou [c], Cristina Tural [d], Miguel Angel Martínez [a,*]

[a] IrsiCaixa, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona (UAB), Badalona, Spain
[b] Infectious Disease Department, Hospital Universitari Germans Trias i Pujol, Badalona, Spain
[c] Fundació LLuita Contra les Infeccions, Badalona, Spain
[d] Internal Medicine Department, Hospital Universitari Germans Trias i Pujol, Badalona, Spain

ARTICLE INFO

Keywords:
HCV
DAA
miRNA
Biomarker
Liver disease

ABSTRACT

In a previous recent work, we recognized three plasma circulating microRNAs (miRNAs)—miR-100-5p_iso3p:-2, miR-122-5p, and miR-192-5p—that correlate largely with liver fibrosis evolution in human immunodeficiency virus type 1 (HIV-1)/hepatitis C virus (HCV) co-infected patients. Here, we investigated whether levels of these three circulating miRNAs can be associated to liver disease evolution in HIV-1/HCV co-infected patients which have achieved HCV sustained virologic response (SVR) 12 weeks after finishing treatment. Eighty-one chronic HIV-1/HCV co-infected patients were longitudinally recruited at baseline (T0) of DAA therapy and 12 weeks (T12) after finishing therapy. At T0 most of the study patients displayed transient elastography values linked to an advanced stage of liver fibrosis (F0-1 9%, F2 11%, F3 32%, F4 48%). Significant reductions in the levels of circulating miR-100-5p_iso3p:-2, miR-122-5p, and miR-192-5p were detected at T12 in SVR patients, in the overall cohort ($P < 0.0001$, $P < 0.0001$, and $P = 0.0008$, respectively) and in patients with advanced (F3-4) liver fibrosis ($p < 0.0001$, $p < 0.0001$, and $P = 0.0011$, respectively). Of note, no significant reduction in the study miRNA levels was found at T12 in patients who did not achieve SVR ($P = 0.8750$, $P = 0.1250$, and $P = 0.1260$, respectively). HCV-cured patients, in contrast to non-responders, significantly reduced their liver stiffness after two years of achieving SVR ($p < 0.0001$). DAA-induced SVR is linked with a significant reduction in circulating levels of miR-100-5p_iso3p:-2, miR-122-5p, and miR-192-5p. Our results indicate that miRNA plasma levels may be a useful biomarker of liver damage progression in HIV-1/HCV co-infected individuals that reach DAA-induced SVR.

1. Introduction

Chronic liver disease is one of the major leading causes of morbidity and mortality in patients infected with human immunodeficiency virus type 1 (HIV-1), due in part to the toxic effects of antiretroviral (ART) treatment and co-infection with hepatitis B or C virus (HCV) [1,2]. The latter accelerates liver disease progression, promoting liver cirrhosis, hepatocellular carcinoma, and death. In

* Corresponding author. Fundació irsiCaixa, Hospital Universitari Germans Trias i Pujol, 08916, Badalona, Spain.
E-mail address: mmartinez@irsicaixa.es (M.A. Martínez).

https://doi.org/10.1016/j.heliyon.2022.e12686
Received 22 August 2022; Received in revised form 21 December 2022; Accepted 21 December 2022
Available online 3 January 2023
2405-8440/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

developed countries, the introduction of HCV treatment with direct-acting antivirals (DAAs) has almost eradicated HCV co-infections in HIV-1 individuals [3]. However, individuals with HCV infection and advanced fibrosis who achieve a sustained virologic response (SVR) after DAA therapy still should be monitored for symptoms of liver disease [4]. Moreover, HIV-1–infected individuals are also affected by the global increase in nonalcoholic fatty liver disease [5,6], which induce liver injury by promoting metabolic disorders such as cardiovascular disease, insulin resistance, and diabetes mellitus [7].

MicroRNAs (miRNAs) are 19–22–nucleotide noncoding RNAs that negatively control cell translation. miRNAs are implicated in many cellular processes, in particular in those involved with the deregulation of gene expression [8]. It is very well known that miRNAs activities are connected with many diseases, including cancer [9], diabetes [10], obesity [11], and cardiovascular disease [12]. Modifications in cellular miRNA levels have been related to specific cell or tissue pathology. These miRNA level changes have allowed the design of new disease diagnosis. Remarkably, miRNAs are secreted into extracellular fluids through extracellular vesicles and delivered to other cells in which they also can perform their biological activities [13-17]. These findings suggest that miRNAs can function as signaling and synchronization entities between cells and organs. Overall, the recent literature highlights miRNAs as excellent disease biomarkers [9–12]. We and others have recently shown that plasma levels of certain miRNAs can be significantly modified in HIV-1/HCV co-infected individuals with liver injuries [18].

Here, we describe the evolution of circulating levels of three miRNAs, miR-100-5p_iso3p:-2, miR-122-5p, and miR-192-5p, which have previously been associated with liver injury in HIV-1/HCV co-infected patients in which HCV infection was eradicated after DAA treatment. Thus, we measured the plasma levels of these miRNAs at therapy baseline and 12 weeks after DAA treatment was completed. We tried here to test the hypothesis that improved liver fibrosis evolution in HCV-cured patients 2 years after virus eradication is signaled by the circulating levels of a number of miRNAs soon (12 weeks) after HCV eradication. Because several single nucleotide polymorphisms (SNPs) have been considered genetic risk factors for liver disease, we assessed three host genetic SNPs, in the genes encoding patatin-like phospholipase domain-containing 3 (PNPLA3), adenosine deaminase acting on RNA-1 (ADAR-1), and interferon-induced helicase C domain-containing 1 (IFIH1), that we had previously found to be associated with advanced liver fibrosis in HIV-1/HCV co-infected patients [18].

Table 1
Clinical characteristics of the study HIV-1/HCV co-infected cohort.

	HIV-1/HCV Study Cohort				
Clinical characteristics	Baseline (T0)	T12	*p*-value[a]	2y_post_T12	*p*-value[b]
N	81	81		67	
Age (y)	52 (49–54.5)	53 (50–55)		54 (51–57)	
Gender (female), N (%)	20 (24.7%)	20 (24.7%)		16 (23.8%)	
Liver stiffness (Kpa)	12 (9.85–18.4)	ND	ND	8.3 (6.2–13.80)	<0.0001
ALT (U/L)	63 (42–84)	21.5 (17–33)	<0.0001	20.5 (16.75–28)	<0.0001
AST (U/L)	66 (48–97)	ND	ND	24 (20.5–32.5)	<0.0001
Platelets ($\times 10^9$/L)	147 (98–175)	148.5 (95–183)	0.4363	161 (116–190)	0.0619
Leucocytes ($\times 10^9$/L)	6.2 (4.55–7.15)	6.2 (4.72–8.25)	0.6031	6.6 (4.9–9.2)	0.0606
Alkaline phosphatase (U/L)	88 (69–119.5)	94.5 (74–111.5)	0.4544	82 (65.75–107.3)	0.2232
Bilirubin (μmol/L)	10.9 (7.89–15.24)	9.53 (6.88–12.81)	0.0697	8.9 (7.5–11.9)	0.0778
Albumin (g/L)	43 (40.4–44.7)	44.2 (42–45.9)	0.0132	43.9 (41.7–45.4)	0.0758
Glucose (mmol/L)	5.01 (4.62–5.48)	5.01 (4.68–5.23)	0.5851	5.1 (4.8–5.6)	0.3729
Total Cholesterol (mmol/L)	3.77 (3.38–4.27)	4.31 (3.82–4.93)	0.0002	4.3 (3.9–5.05)	0.0001
LDL (mmol/L)	2.02 (1.61–2.47)	2.48 (2.02–3.12)	<0.0001	2.6 (1.92–3.2)	<0.0001
HDL (mmol/L)	1.11 (0.91–1.26)	1.08 (0.95–1.29)	0.9980	1.1 (0.9–1.3)	0.7901
Triglycerides (mmol/L)	1.37 (1–1.84)	1.21 (0.90–1.64)	0.1681	1.3 (1–1.8)	0.8682
HCV viral load (log10 IU/ml)	6.4 (5.8–6.9)				
HCV genotype, N (%)					
1	50 (61.7%)				
3	4 (4.9%)				
4	27 (33.3%)				
Liver fibrosis stage (Metavir score), N (%)					
F0F1	7 (8.6%)	ND		27 (40.3%)	
F2	9 (11.1%)	ND		15 (22.4%)	
F3	26 (32.1%)	ND		4 (5.9%)	
F4	39 (48.1%)	ND		21 (31.3%)	<0.00001
CD4+T (cells/μl)	606 (382–764)	566 (411–850)	0.9840	617 (468.5–901)	0.4532
CD8+T (cells/μl)	784 (458–1074)	781.5 (545–1102)	0.5179	911 (487–1266)	0.1408
CD4/CD8 ratio	0.89 (0.52–1.31)	0.85 (0.50–1.14)	0.6371	0.87 (0.52–1.23)	0.9941

[a] p-value (T0-T12).
[b] p-value (T0-2years_post_T12). ND, not determined.

2. Methods

2.1. Study design and patient population

A total of 81 co-infected HIV-1/HCV patients were enrolled in a longitudinal study of HCV treatment with six different DAA combination drugs, according to European Association for the Study of the Liver (EASL) clinical guidelines. Depending on the DAA combination, patients were treated for 12 or 24 weeks with or without ribavirin (RBV). Treatment was performed between December 2014 and December 2015. The six DAA treatment combinations were classified as sofosbuvir (SOF) or no-SOF treatments. The SOF treatments were SOF plus simeprevir (SMV) ± RBV (n = 26), SOF plus daclatasvir (DCV) ± RBV (n = 3), and Harvoni (SOF and ledipasvir) ± RBV (n = 20). No-SOF treatments were Viekirax (ombitasvir, paritaprevir, and ritonavir) plus Exviera (dasabuvir) ± RBV (n = 20), Viekirax ± RBV (n = 11), and SMV plus DCV (n = 1). The primary goal of HCV treatment is to cure HCV infection by achieving an SVR, defined as undetectable plasma HCV-RNA load 12 weeks after therapy completion. Two plasma samples were collected, at baseline (T0) and 12 weeks after the treatment was completed (T12). To study liver fibrosis evolution after curing HCV with DAA, liver stiffness (kPa) was measured by transient elastography (FibroScan) at T0 and 2 years after T12. The Metavir scores utilized in the study were F01 < 7.1 kPa, F2 = 7.1–9.5 kPa, F3 = 9.5–12.5 kPa, and F4 >12.5 kPa. Virological and clinical data (HIV-1 $CD4^+$ and $CD8^+$ T-cell counts, HIV-1 viral load, HCV genotype, HCV viral load, albumin, transaminase levels, total cholesterol, low-density and high-density lipoprotein, and platelets) were determined by conventional methods (Table 1) at three time points: T0, T12, and 2 years after T12. As a control group, 21 plasma samples from a cohort of healthy uninfected volunteers was analyzed. All study participants were informed of this study and gave their written consent. The ethics committee of the Germans Trias i Pujol University Hospital approved the study protocol.

2.2. RNA isolation

RNA was purified from 100 μL of centrifuged plasma (5 min at 3000*g*) using the MagMAX mirVana Total RNA Isolation Kit (Thermo Fisher Scientific). RNA was then resuspended in 50 μL of elution buffer previously heated at 60 °C.

2.3. Reverse transcription quantitative real-time PCR (RT-qPCR)

Two μL of total extracted RNA was reverse transcribed in two steps. First, a poly A tail was added by using 0.25 μL (1.25 U) of *E. coli* poly A polymerase (5000 U/mL) (New England Biolabs), 1 μL of 10× poly A buffer (New England Biolabs), 1 μL of 10 mM ATP and 7.75 μL of RNase free water. This final mix (10 μL) was then incubated 30 min at 37 °C. As an internal control, we added 10^5 copies of miR-RNU6B, which is not found in our human plasma samples. Next, polyadenylated miRNAs were reverse-transcribed by adding the former total 10 μL (poly A tailing reaction) into the RT reaction that included 4 μL of 5× MMLUV-RT buffer (Invitrogen), 1 μL of M-MuLV-RT (Invitrogen), 2 μL of 10 μM RT-primer, 0.5 μL of 40U/μL RNasin (Promega), 0.4 μL of 25 mM dNTPs, 2 μL of 0.1 M DTT, and 0.1 μL of RNAse-free water. These 20 μL were incubated 50 min at 37 °C and then inactivated at 70 °C for 15 min. The RT oligonucleotide was 5′-CAGGTCCAGTTTTTTTTTTTTTTVN-3′. To determine miRNAs total copy number, 1 μL of the above cDNA was added to 0.625 μL (250 nM) of each miRNA's specific DNA oligonucleotides (forward and reverse), 12.5 μL of 2× SYBR Green PCR Master mix (Thermo Fisher Scientific) and 10.25 μL of water. The corresponding forward oligonucleotides for miR-100-5p_iso3p:-2, miR-122-5p, and miR-192-5p were 5′-CAGAACCCGTAGATCCGA-3′, 5′-GCAGTGGAGTGTGACAATG-3′, and 5′-CAGCTGACCTAT-GAATTGACA-3′, respectively. Similarly, reverse oligonucleotides were 5′-GGTCCAGTTTTTTTTTTTTTTCAAGT-3′, 5′-CCAGTTTTTTTTTTTTTTTCAAACACC-3′, and 5′-TCCAGTTTTTTTTTTTTTTTGGCT-3′, respectively. RT-qPCRs were carried out in triplicate in a 96-well reaction plate. In parallel with the specific miRNA reaction a control RNU6B standard curve was also performed. RNU6B forward and reverse oligonucleotides were respectively the following: 5′-ACACGCAAATTCGTGAAGCGTTCCA-3′ and 5′-CAGGTCCAGTTTTTTTTTTTTTTAAA-3′. The absolute miRNA qPCR amplification was carried out using the standard protocol for SYBR GREEN reactions (Thermo Fisher Scientific) in a Quantstudio 5.0 real-time PCR system (Thermo Fisher Scientific). The qPCR program was: 2 min at 50 °C followed by 10 min at 95 °C, 40 cycles of 15 s at 95 °C plus 1 min at 60 °C, and a final dissociation stage (15 s at 95 °C, 1 min at 60 °C, and 1 s at 95 °C). To ensure amplification specificity, the melting curve and the result from the dissociation stage were employed in each run. The Quantstudio Design and Analysis software (Thermo Fisher Scientific) was used to automatically calculate miRNA total copy number which was then calculated by extrapolating the threshold cycle (Ct) taken for each sample on the standard curve. To each 96-well plate we added control samples. There were two positive controls. First, one plasma sample for one patient was used as an internal positive control in all the reactions. Second, to confirm the detection on the standard curve we added 10^4 copies of the cDNA derived from each miRNA synthetic oligonucleotide. Finally, 10^4 copies of the other miRNAs and the control RNU6B were tested as negative controls to guarantee that there was no cross-amplification.

Study miRNA-specific DNA primers (forward and reverse) were designed using the miRprimer software. Based on the sequence of each miRNA, the specific miRNA standard curves and the corresponding cDNA template were performed by using 10 ng of a RNase-free HPLC purified synthetic RNA oligonucleotide (IDT DNA technologies) (RNU6B [5′-CGCAAGGAUGACACGCAAAUUCGU-GAAGCGUUCCAUAUUUUU-3′], miRNA-100-5p [5′-AACCCGUAGAUCCGAACUUGUG-3′], miRNA-122-5p [5′-UGGAGUGUGA-CAAUGGUGUUUG-3′] and miRNA-192-5p [5′-CUGACCUAUGAAUUGACAGCC-3′]. Ten-fold dilutions (10^1–10^7) in 1× TE buffer (IDT DNA technologies) were performed to generate cDNA standard curves. Dilutions were aliquoted (8 μL) to avoid freeze-and-thaw cycles and stored at −30 °C.

2.4. SNP genotyping

QuickExtract DNA Extraction 1.0 solution (Epicentre/Lucigen) was used to extract genomic DNA from peripheral blood mononuclear cells (PBMCs). SNP genotyping (Applied Biosystems, Thermo Fisher Scientific) was performed with 2 μL of the extracted DNA. QuantStudio 5.0 real-time PCR system and TaqMan assays (Applied Biosystems, Thermo Fisher Scientific) were used to genotyoe the three study SNPs, PNPLA3, ADAR1 and IFIH1 (reference numbers rs738409, C____7241_10, rs1127313, C__8724398_10, and rs1990760, C__2780299_30, respectively).

2.5. Statistical analysis

Paired samples (e.g., T0/T12) were compared by using Wilcoxon's match-paired test and the Mann Whitney *t*-test. Pearson's correlation analysis to associate miRNA expression with clinical, virological, and liver fibrosis parameters was determined. Association analysis between liver fibrosis and each SNP was fitted under log-additive models. Univariate, multivariate, and multinomial logistic regression analysis were performed to associate liver fibrosis progression and liver damage with the different patient risk factors. The logistic regression analysis was carried out by using liver cirrhosis (stage F4) as dependent variable. In the univariate analysis all baseline patient clinical parameters were included as covariates. In contrast, the multivariate analysis was adjusted for sex and only those covariates with a level of statistical significance of $P < 0.05$ in the univariate analysis were included. Results were expressed as the odds ratio (OR) and 95% confidence interval (95% CI). Multivariate and multinomial logistic regression models with a better Akaike information criterion (AIC) were chosen. To avoid multicollinearity, in the multiple or multinomial logistic regression models highly correlated variables were not included. Statistical analyses were performed with GraphPad Prism 8.0 (GraphPad Software).

3. Results

3.1. Characteristics of the HIV-1/HCV co-infected patient cohort

We retrospectively studied a longitudinal cohort of 81 HIV-1/HCV co-infected patients treated with DAA. Transient elastography at treatment baseline (T0) indicated that most of the study patients were in an advanced stage of liver fibrosis (F0-1 9%, F2 11%, F3 32%, F4 48%) (Table 1). A high percentage of patients, 63%, were infected with HCV genotype 1, followed by 32% with genotype 4, and 5% with genotype 3. At the beginning of treatment all patients were on ART and had undetectable levels of HIV-1 RNA. A univariate logistic regression analysis at T0 revealed that cirrhosis (F4) was significantly associated with plasma albumin ($P = 0.0024$), platelet counts ($P < 0.0001$), total cholesterol ($P = 0.0382$), leucocyte counts ($P = 0.0030$), and $CD4^+$ and $CD8^+$ T-cell counts ($P = 0.0496$ and $P = 0.0062$, respectively) (Table 2). A multivariate analysis confirmed the significance of albumin ($P = 0.0269$) and platelet counts ($P = 0.0081$) with the presence of cirrhosis at T0 (Table 2). After DAA treatment, SVR at week 12 was observed in 77 patients (95%). Four cirrhotic patients (F4), two of them infected with HCV of genotype 1 and two with genotype 4, treated with the SOF/SMV combination did not respond to therapy and had not reduced their plasma levels of HCV RNA 12 weeks after finishing their treatment (6.1 vs. 5.7 $\log_{10}$ IU/mL, $P = 0.3412$). At 12 weeks (T12) after the conclusion of treatment, a significant decrease in alanine transaminase (ALT) ($P < 0.0001$) and significant increases in albumin ($P = 0.0132$), total cholesterol ($P = 0.0002$), and low-density lipoprotein (LDL) (p <

Table 2
Univariate and multivariate analysis of the factors associated with liver cirrhosis (F4) at baseline (T0).

	Univariate			Multivariate[a]		
Baseline (T0) characteristics	OR	95% CI	p-value	OR	95% CI	p-value
Gender	4.625	1.57 to 15.76	0.0082			
ALT (U/L)	0.993	0.98 to 1.003	0.2002			
AST (U/L)	1.005	0.99 to 1.019	0.4216			
Albumin (g/L)	0.803	0.69 to 0.916	0.0024	0.771	0.59 to 0.956	0.0269
Platelets ($\times 10^9$/L)	0.976	0.96 to 0.986	<0.0001	0.976	0.96 to 0.992	0.0081
Total Cholesterol (mmol/L)	0.543	0.29 to 0.947	0.0382			
LDL (mmol/L)	0.541	0.27 to 1.030	0.0708			
HDL (mmol/L)	0.677	0.18 to 2.374	0.5448			
Triglycerides (mmol/L)	0.918	0.51 to 1.599	0.762			
Leucocytes (x10^9/L)	0.682	0.52 to 0.859	0.003			
CD4+T (cells/μl)	0.999	0.99 to 0.999	0.0496			
CD8+T (cells/μl)	0.998	0.996 to 0.999	0.0062			
CD4/CD8 ratio	1.679	0.75 to 3.959	0.2158			
miR100-5p_iso3p:-2 (log10 copies/μl)	1.5	0.74 to 3.132	0.263			
miR122-5p (log10 copies/μl)	1.141	0.64 to 2.075	0.6549			
miR192-5p (log10 copies/μl)	2.339	1.12 to 5.292	0.0299	4.113	1.46 to 14	0.0124
IFIH1_rs1990760 C/T (TT = 0; CT = 1; CC = 2)	0.755	0.38 to 1.460	0.4071			
ADAR1_rs1127313 (AA = 0; AG = 1; GG = 2)	2.183	1.08 to 4.711	0.0357			
PNPLA3_rs738409 (CC = 0; CG = 1; GG = 2)	1.158	0.53 to 2.554	0.7133			
IFNL3_rs12979860 (CC = 0; CT = 1; TT = 2)	0.878	0.42 to 1.801	0.7222			

[a] Multivariate analysis adjusted by gender, platelets, albumin, CD4+T, ALT, miR192-5p, ADAR1_rs1127313.

0.0001) were observed (Table 1). Remarkably, 2 years after finishing DAA treatment, except patients infected with HCV of genotype 3, a significant decrease in liver fibrosis ($P < 0.0001$) was observed in SVR patients (Fig. 1), but not in non-responders ($P = 0.5000$). Also remarkable is that 53% of the patients who had liver fibrosis F3-4 at T0 and who achieved SVR reduced their fibrosis to F0-1 levels after 2 years of no presence of HCV. Liver fibrosis reduction after SVR was significant in individuals infected with HCV genotype 1 ($P < 0.0001$) and genotype 4 ($P = 0.0040$), but not in the three individuals infected with HCV genotype 3 ($P = 0.5000$). SVR patients also had significant reductions in their levels of ALT ($P < 0.0001$) and aspartate transaminase ($P < 0.0001$), and significant increases in total cholesterol ($P = 0.0001$) and LDL ($P < 0.0001$) after 2 years of absence of HCV infection. During the study period, all patients maintained undetectable levels of HIV-1 RNA, 2 developed cardiovascular diseases and 2 developed non-liver associated carcinomas; after finishing the study period, 2 non-responder patients developed hepatocellular carcinoma and another 4 other types of cancers.

3.2. Reduction of circulating levels of miRNAs after DAA treatment

Using next-generation sequencing to analyze plasma small RNA profiles, we have previously identified a signature of three miRNAs that were highly correlated with progression to liver fibrosis in HIV-1/HCV co-infected patients. This signature included the miRNAs miR-100-5p, miR-122-5p, and miR-192-5p [19]. In order to validate the utility of above miRNA signature, we used the commercially available TaqMan miRNA assay to quantify its expression in plasma. Although miR-122-5p and miR-192-5p were accurately quantified, the TaqMan miRNA assay was unable to properly quantify miR-100-5p [20]. The miR-100-5p sequence analysis of the study plasma samples from HIV-1/HCV co-infected individuals revealed that the canonical variant was almost absent. In contrast, miR-100-5p_iso_3p:-2, a miR-100-5p isomer variant, was the most abundant miR-100-5p sequence, tailed by miR-100-5p_iso_3p:-1. These results strongly suggested that the absence of the miR-100-5p canonical sequence from our study samples was the reason why the TaqMan assay was unable to detect miR-100-5p. While sequence analysis revealed that the miR-122-p and miR-192-5p canonical variants were abundantly detected, suggesting, again, why the TaqMan assay was able to detect and acutely quantify these two miRNAs. In order to precisely quantify the expression of miR-100-5p, we adjusted a previously described SYBR-green-based RT-qPCR method to measure miR-100-5p_iso_3p:-2, miR-122-5p, and miR-192-5p expression [21]. We have previously demonstrated that this adapted SYBR-green RT-qPCR protocol allows the plasma level quantification of different miRNA isomers (isomiRNAs) and canonical miRNAs [20].

To determine whether miRNA expression was affected by DAA treatment, we compared the circulating levels of miR-100-5p_iso3p:-2, miR-122-5p, and miR-192-5p between two time points, T0 and T12. At T0, individuals infected with HCV genotype 4 presented

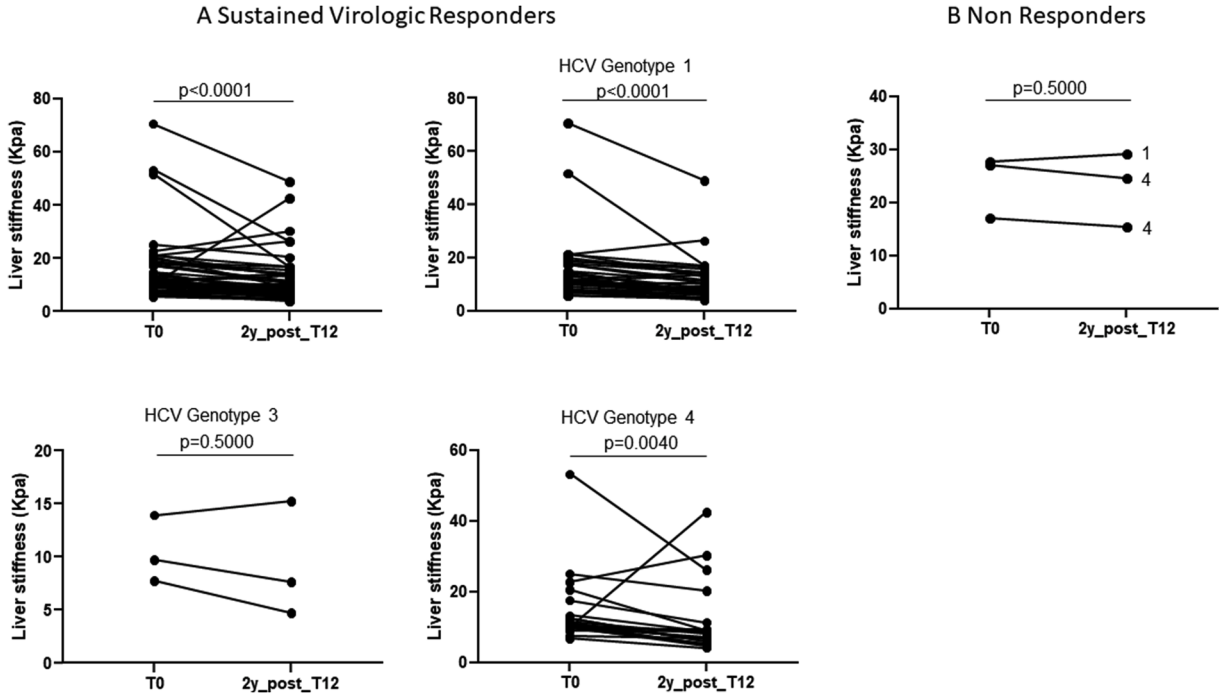

Fig. 1. Evolution of liver fibrosis 2 years after completion of HCV DAA therapy. (A) A significant decrease in liver fibrosis ($p < 0.0001$) was observed in the HIV-1/HCV co-infected patients in whom HCV was eradicated after DAA treatment (SVRs). This significant liver fibrosis reduction was observed in HCV genotype 1 or genotype 4 infected individuals. **(B)** Significant liver fibrosis reduction was not detected in the group of non-responders. The three nonresponder patients with liver fibrosis data 2 years after completion of DAA therapy two were infected with HCV genotype 4 and one with genotype 1. Liver fibrosis stiffness (kPa) was measured by transient elastography (FibroScan). The Metavir scores used in this study were F01 < 7.1 kPa, F2 = 7.1–9.5 kPa, F3 = 9.5–12.5 kPa, and F4 >12.5 kPa.

significantly higher levels of miR-100-5p_iso_3p:-2 ($P = 0.0144$) and miR-122-5p ($P = 0.0004$). No significant dissimilarities were observed in the expression levels of miR192-5p ($P = 0.1235$) between HCV genotypes. When samples were stratified by sex, no miRNA level differences were found at T0 ($P = 0.9365$, $P = 0.5956$, $P = 0.9315$, respectively). We also analyzed whether T0 miRNA levels were associated with clinical parameters related to liver injury. Patients with higher levels of ALT (>41 U/L) had significantly higher levels of miR-100-5p_iso3p:-2 ($P = 0.0302$) and miR-122-5p ($P = 0.0017$), but not of miR192-5p ($P = 0.2080$). Although modest, significant correlations were reached at T0 between ALT and miR-100-5p_iso3p:-2 levels ($r = 0.27$, $P = 0.0141$) and miR-122-5p levels ($r = 0.31$, $P = 0.0045$). Similarly, patients with high triglyceride levels (>1.7 nmol/L) had significantly higher levels of miR-100-5p_iso3p:-2 ($P = 0.0092$) and miR-122-5p ($P = 0.0098$), but not of miR-192-5p ($P = 0.0780$). At T0, triglycerides also correlated with miR-100-5p_iso3p:-2 levels ($r = 0.22$, $P = 0.0473$) and miR-122-5p levels ($r = 0.25$, $P = 0.0264$). At T0, the lipid parameters high total cholesterol (>3.7 nmol/L) and high LDL (>2.0 nmol/L) correlated with lower levels of miRNA-192-5p ($P = 0.0260$ and $P = 0.0037$, respectively), but not of miR-100-5p_iso3p:-2 ($P = 0.2501$ and $P = 0.0565$, respectively) and miR-122-5p ($P = 0.1900$ and $P = 0.0571$, respectively). A meaningful negative correlation was identified between the levels of miR-192-5p and the levels of LDL ($r = -0.25$, $P = 0.0278$). Although a tendency for higher amounts of miR-100-5p_iso3p:-2, miR-122-5p, and miR-192-5p was found at T0 in patients with significant liver fibrosis (F3-4), these values did not reach significance ($P = 0.1976$, $P = 0.1377$, and $P = 0.0899$, respectively) (Fig. 2). Univariate and multivariate logistic regression analysis indicated that at T0, miR-192-5p plasma levels were significantly associated with cirrhosis ($P = 0.0299$ and $P = 0.0124$, respectively) but the levels of miR-100-5p_iso3p:-2 and miR-122-5p were not (Table 2). Finally, the association between levels of $CD4^+$ T cells with miRNA expression levels at T0 was explored. Intriguingly, patients with high $CD4^+$ T-cell counts (>500 cells/μl) had significantly higher levels of miR-100-5p_iso3p:-2 ($P = 0.0065$) and miR-122-5p ($P = 0.0033$), but not of miR-192-5p ($P = 0.1350$). A significant positive correlation was also found between T0 $CD4^+$ T-cell counts and levels of miR-100-5p_iso3p:-2, miR-122-5p, and miR192-5p ($r = 0.30$, $P = 0.0086$, $r = 0.35$, $P = 0.0015$ and $r = 0.25$, $P = 0.0289$, respectively).

Importantly, SVR patients at T12 had significantly reduced levels of the three tested miRNAs, miR-100-5p_iso3p:-2, miR-122-5p, and miR-192-5p ($P < 0.0001$, $P < 0.0001$, and $P = 0.0008$, respectively) (Fig. 3A). These reduced levels were similar to those seen in a cohort of control uninfected individuals. This reduction was also observed in individuals with advanced (F3-4) liver fibrosis ($p < 0.0001$, $p < 0.0001$, and $P = 0.0011$, respectively). In contrast, no significant reduction was observed in individuals who did not achieve SVR ($P = 0.8750$, $P = 0.1250$, and $P = 0.1260$, respectively) (Fig. 3B). The stratification of SVR patients by DAA treatment also showed that, at T12, patients with SOF-based treatments had significantly lower median levels of miR-100-5p_iso3p:-2 (median [IQR], 167 [50–445] vs. 60 [37–116.5]; $P = 0.0005$) and miR-192-5p (median [IQR], 336 [144–873] vs. 134.5 [67–280.8]; $P = 0.0021$). No significant differences were detected in the circulating levels of miR-122-5p (median [IQR], 919.5 [256.8–3802] vs. 673 [310.5–1004]; $P = 0.2718$).

3.3. Circulating levels of miRNAs associated with host genetic markers for liver injury

We have previously demonstrated that SNPs in PNPLA3 (rs738409), ADAR-1 (rs1127313), and IFIH1 (rs1990760) are associated with advanced liver fibrosis in HIV-1/HCV co-infected patients [6]. We aimed here to know how these genetic polymorphisms impacted the plasma levels of our three study miRNAs. Univariate logistic regression analysis revealed that in our study cohort only ADAR-1 (rs1127313) was significantly associated at T0 with the presence of liver cirrhosis (Table 2). Patients with the PNPLA3_CG + GG (I184 M) genotype had significantly higher levels of miR-100-5p_iso3p:-2 ($P = 0.0121$) and miR-122-5p ($P = 0.0008$), but not of miR-192-5p ($P = 0.0849$) (Fig. 4). Confirming these results, we also found a significant positive correlation of the PNPLA3 (I184 M) genotype with the levels of miR-100-5p_iso3p:-2 ($r = 0.26$, $P = 0.0219$) and miR-122-5p ($r = 0.36$, $P = 0.0011$).

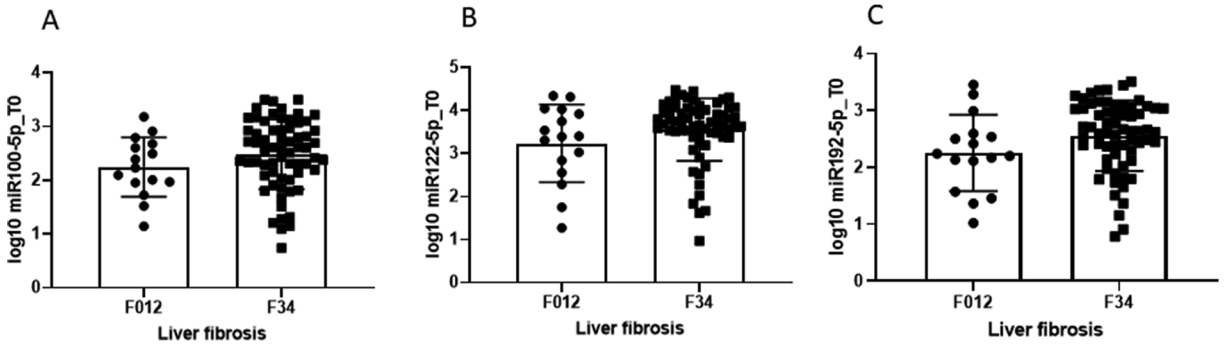

Fig. 2. Baseline (T0) association between plasma levels of miR-100-5p_iso3p:-2, miRNA-122-5p, and miRNA-192-5p and elastography liver fibrosis scores. While a tendency for higher levels of **(A)** miR-100-5p_iso3p:-2, **(B)** miR-122-5p, and **(C)** miR-192-5p was found at T0 in patients with significant liver fibrosis (F3-4), these differences did not reach significance.

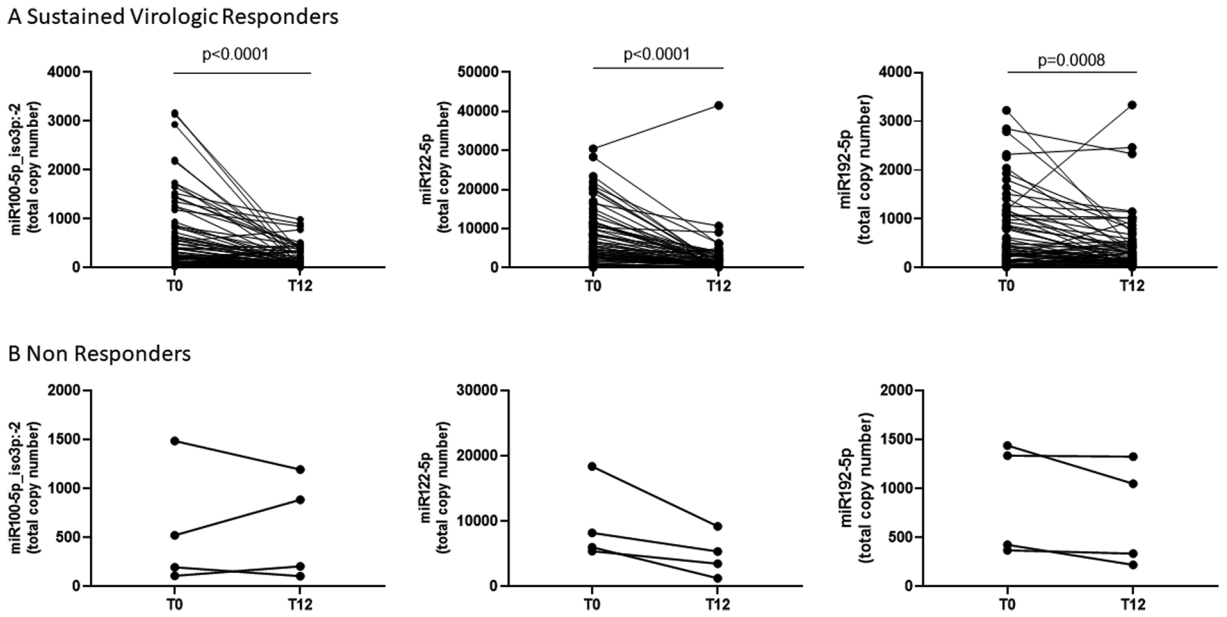

Fig. 3. Evolution of plasma levels of miRNAs miR-100-5p_iso3p:-2, miRNA-122-5p, and miRNA-192-5p 12 weeks after completion of HCV DAA therapy. (A) A significant reduction in the levels of the three tested miRNAs was observed in the HIV-1/HCV co-infected patients that eradicated HCV after DAA treatment (SVRs). **(B)** No significant reduction was observed in individuals that did not achieve SVR 12 weeks after stopping DAA therapy.

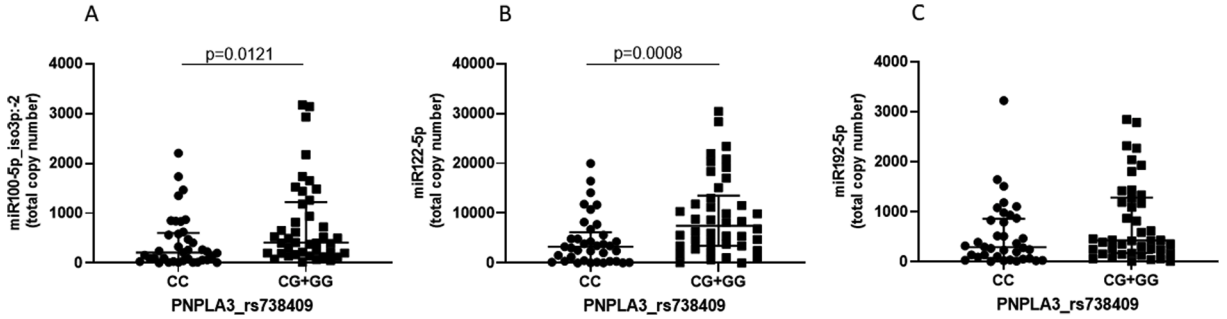

Fig. 4. Baseline (T0) associations of plasma levels of miR-100-5p_iso3p:-2, miRNA-122-5p, and miRNA-192-5p with the patatin-like phospholipase domain-containing 3 (PNPLA3) I148M variant polymorphism. Significantly higher plasma levels of the three tested miRNAs, **(A)** miR-100-5p_iso3p:-2, **(B)** miRNA-122-5p and **(C)** miRNA-192-5p, were observed at T0 in individuals carrying the PNPLA3 I148 M variant polymorphism.

4. Discussion

Previous work has recognized the utility of measuring circulating plasma levels of miRNAs as noninvasive biomarkers for establishing diverse stages of liver injury [22-24], especially among patients infected with HCV or/and HIV-1 [18,25,26]. These studies indicate that circulating miRNA levels correlate with liver activity tests and are independent forecasts of liver disease. In particular, levels of miRNA-122 and miRNA-192 have been related to liver disease in HIV-1/HCV co-infected patients [18,26,27]. Moreover, we have recently identified in HIV-1/HCV-co-infected individuals a plasma signature of circulating miRNAs miR-100-5p_iso3p:-2, miRNA-122-5p, and miRNA-192-5p levels that is highly associated with liver fibrosis development [19]. However, few data exist regarding the evolution of these circulating liver disease–associated miRNAs after the successful eradication of HCV through DAA-based therapies. In this study, we explored the fate of the circulating levels of miR-100-5p_iso3p:-2, miRNA-122-5p, and miRNA-192-5p in plasma from a group HIV-1/HCV co-infected patients treated with HCV DAAs.

We found that HCV eradication has a strong impact on circulating miRNAs in HIV-1/HCV co-infected patients. A significant reduction in circulating levels of liver disease–associated miRNAs miR-100-5p_iso3p:-2, and miR-192-5p was found after successful DAA, supporting the notion that the profile of circulating miRNAs in the HCV SVR individuals is the result of HCV eradication. The observed reduction was deeper in patients treated with SOF-based regimens. This reduction of plasma levels of the studied miRNAs was

accompanied by a reduction in the liver fibrosis stage 2 years after the eradication of HCV infection, indicating that miRNAs may provide a valuable surrogate biomarker of liver fibrosis development in individuals co-infected with HCV/HIV-1. These findings extend prior studies regarding the applicability of plasma miRNA levels as biomarkers for the early diagnosis, prognosis, and estimation of liver injury [6,18,19,22–27]. Our findings also confirm that DAA therapy appears to impact not only HCV replication but also systemic cell-cell communication, by modifying the circulating levels of a number of miRNAs [13].

Prior work performed with extracellular vesicles purified from plasma of healthy donors and HCV-infected patients before and after DAA treatment delivered somehow an opposite final output of the therapy [17]. In this report, SVR does not restore extracellular vesicle antifibrogenic miRNA cargo, including miRNA-122-5p, suggesting that the risk of liver disease progression remains in some HCV patients even after virological cure. Since our study approach was not restricted to the analysis of extracellular vesicles but included the characterization of total small RNA present in circulating plasma, it remains to be elucidated whether these two methodologies might be comparable. Of note, miRNA-122-5p and miRNA-192-5p have been found loaded in circulating extracellular vesicles [15-17]. In addition, the impact of HIV-1 coinfection in our study cohort can not be discarded.

PNPLA3/rs738409 is among the strongest genetic risk factors for nonalcoholic fatty liver disease/non-alcoholic steatohepatitis, liver cirrhosis, and hepatocellular carcinoma [28-30] as well being significantly associated with liver fibrosis in HIV-1/HCV co-infected patients (6). PNPLA3/rs738409 may promote fibrosis development by activating specific fibrogenic pathways [31]. Our finding that, before DAA treatment, the PNPLA3 I184 M genotype is associated with significantly higher levels of miR-100-5p_iso3p:-2 and miR-122-5p is in line with the association of this genetic marker and liver injury. Similarly, we confirmed our previous findings about the association of the ADAR-1/rs1127313 genetic marker and liver fibrosis in HIV-1/HCV co-infected individuals [6,32]. miR-100-5p, miRNA-122-5p, and miRNA-192-5p have been associated with histological liver disease severity, nonalcoholic fatty liver disease/non-alcoholic steatohepatitis, and hepatocellular carcinoma [18,19,24,26,33–39]. Our analysis of viremic HCV patients showed a positive correlation between circulating levels of miR-100-5p_iso3p:-2 and miRNA-122-5p and ALT and triglycerides. Likewise, lipid parameters such as high total cholesterol and high LDL were associated with lower plasma levels of miRNA-192-5p. These results concur with previous studies in which HCV-cured patients had increased levels of total cholesterol and LDL, which may suggest an augmentation of risks of cardiovascular events and liver fat accumulation [40-42]. Interestingly, in our patients a greater increase in cholesterol was also signaled by a reduction in plasma miR-192-5p levels.

Overall, our results confirm the relevance of the three studied miRNAs in signaling liver injury in HCV-infected patients. Since SVR is accompanied by a normalization of the study miRNAs, our results may have translational relevance for individuals with an unfavorable miRNA profile after DAA therapy, because they may obtain a profit from longer DAA therapy or more precise monitoring of liver disease progression and immune restoration. As previously suggested, persistent fibrogenic signals in HCV individuals treated with DDAs after SVR would support the long-term supervising and clinical management of these individuals [4].

While our findings are limited to three miRNAs, and additional studies should be done to extend these results to other circulating plasma markers, these miRNAs provide a simple, cost-effective approach to monitor liver disease progression in HCV-cured patients. Another limitation of our study is the absence liver stiffness data at T12. Liver stiffness decreases rapidly after obtaining SVR due mainly to the reduction of hepatic inflammation while its reduction tends to reach a plateau few months after the end of therapy. Nevertheless, increasing our knowledge regarding the specific miRNAs differential expression in diverse pathologies and physiological situations together with functional studies should allow us the discovery of better and new biomarkers of disease progression.

Financial support

This work was supported by the Spanish Instituto de Salud Carlos III (PI18/00157). We thank Foundation Dormeur for financial support for the acquisition of the QuantStudio 5 real-time PCR system.

Declaration of competing interest

The authors declare no conflicts of interest.

References

[1] J.C. Price, C.L. Thio, Liver disease in the HIV-infected individual, Clin. Gastroenterol. Hepatol. 8 (2010) 1002–1012, https://doi.org/10.1016/J.CGH.2010.08.024.

[2] E.C. Verna, Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in patients with HIV, Lancet. Gastroenterol. Hepatol. 2 (2017) 211–223, https://doi.org/10.1016/S2468-1253(16)30120-0.

[3] M.A. Martinez, S. Franco, Discovery and development of antiviral therapies for chronic hepatitis C virus infection, Adv. Exp. Med. Biol. 1322 (2021) 139–157, https://doi.org/10.1007/978-981-16-0267-2_6.

[4] M.A. Martinez, C. Tural, S. Franco, Circulating MicroRNAs as a tool for diagnosis of liver disease progression in people living with HIV-1, Viruses 14 (2022) 1118, https://doi.org/10.3390/V14061118. Page 1118.

[5] L. Soldevila, M. Tenesa, J. Horneros, J. Bechini, J.J. López, R. Pérez, M.À. Martínez, D. Ouchi, S. Franco, N. Perez-Àlvarez, D. Buccione, B. Clotet, C. Tural, Association between visceral abdominal fat accumulation and severity of liver fibrosis in nondiabetic individuals coinfected by human immunodeficiency virus and hepatitis C virus, AIDS Res. Hum. Retrovir. 36 (2020) 205–213, https://doi.org/10.1089/AID.2019.0097.

[6] S. Franco, J. Horneros, L. Soldevila, D. Ouchi, I. Galván-Femenía, R. de Cid, M. Tenesa, J. Bechini, R. Perez, J.M. Llibre, B. Clotet, C. Tural, M.A. Martínez, Single nucleotide polymorphisms in PNPLA3, ADAR-1 and IFIH1 are associated with advanced liver fibrosis in patients co-infected with HIV-1//hepatitis C virus, AIDS 35 (2021) 2497–2502, https://doi.org/10.1097/QAD.0000000000003066.

[7] T. Barré, C. Protopopescu, F. Bani-Sadr, L. Piroth, T. Rojas Rojas, D. Salmon-Ceron, L. Wittkop, L. Esterle, P. Sogni, K. Lacombe, J. Chas, O. Zaegel, M.L. Chaix, P. Miailhes, L. Serfaty, F. Marcellin, M.P. Carrieri, Elevated fatty liver index as a risk factor for all-cause mortality in human immunodeficiency virus-hepatitis C virus-coinfected patients (ANRS CO13 HEPAVIH cohort study), Hepatology 71 (2020) 1182–1197, https://doi.org/10.1002/HEP.30914.

[8] S.L. Ameres, P.D. Zamore, Diversifying microRNA sequence and function, Nat. Rev. Mol. Cell Biol. 14 (2013) 475–488, https://doi.org/10.1038/NRM3611.

[9] P.S. Mitchell, R.K. Parkin, E.M. Kroh, B.R. Fritz, S.K. Wyman, E.L. Pogosova-Agadjanyan, A. Peterson, J. Noteboom, K.C. O'Briant, A. Allen, D.W. Lin, N. Urban, C.W. Drescher, B.S. Knudsen, D.L. Stirewalt, R. Gentleman, R.L. Vessella, P.S. Nelson, D.B. Martin, M. Tewari, Circulating microRNAs as stable blood-based markers for cancer detection, Proc. Natl. Acad. Sci. U. S. A 105 (2008) 10513–10518, https://doi.org/10.1073/PNAS.0804549105.

[10] C. Guay, E. Roggli, V. Nesca, C. Jacovetti, R. Regazzi, Diabetes mellitus, a microRNA-related disease? Transl. Res. 157 (2011) 253–264, https://doi.org/10.1016/J.TRSL.2011.01.009.

[11] V. Capobianco, C. Nardelli, M. Ferrigno, L. Iaffaldano, V. Pilone, P. Forestieri, N. Zambrano, L. Sacchetti, miRNA and protein expression profiles of visceral adipose tissue reveal miR-141/YWHAG and miR-520e/RAB11A as two potential miRNA/protein target pairs associated with severe obesity, J. Proteome Res. 11 (2012) 3358–3369, https://doi.org/10.1021/PR300152Z.

[12] A. Caroli, M.T. Cardillo, R. Galea, L.M. Biasucci, Potential therapeutic role of microRNAs in ischemic heart disease, J. Cardiol. 61 (2013) 315–320, https://doi.org/10.1016/J.JJCC.2013.01.012.

[13] L. Santangelo, V. Bordoni, C. Montaldo, E. Cimini, A. Zingoni, C. Battistelli, G. D'Offizi, M.R. Capobianchi, A. Santoni, M. Tripodi, C. Agrati, Hepatitis C virus direct-acting antivirals therapy impacts on extracellular vesicles microRNAs content and on their immunomodulating properties, Liver Int. 38 (2018) 1741–1750, https://doi.org/10.1111/LIV.13700.

[14] H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J.J. Lee, J.O. Lötvall, Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (2007) 654–659, https://doi.org/10.1038/NCB1596.

[15] J.H. Kim, C.H. Lee, S.W. Lee, Exosomal transmission of MicroRNA from HCV replicating cells stimulates transdifferentiation in hepatic stellate cells, Mol. Ther. Nucleic Acids 14 (2019) 483–497, https://doi.org/10.1016/J.OMTN.2019.01.006.

[16] G. Chiabotto, C. Pasquino, G. Camussi, S. Bruno, Molecular pathways modulated by mesenchymal stromal cells and their extracellular vesicles in experimental models of liver fibrosis, Front. Cell Dev. Biol. 8 (2020), https://doi.org/10.3389/FCELL.2020.594794.

[17] C. Montaldo, M. Terri, V. Riccioni, C. Battistelli, V. Bordoni, G. D'Offizi, M.G. Prado, F. Trionfetti, T. Vescovo, E. Tartaglia, R. Strippoli, C. Agrati, M. Tripodi, Fibrogenic signals persist in DAA-treated HCV patients after sustained virological response, J. Hepatol. 75 (2021) 1301–1311, https://doi.org/10.1016/J.JHEP.2021.07.003.

[18] S. Franco, D. Buccione, R. Pluvinet, B. Mothe, L. Ruiz, M. Nevot, A. Jordan-Paiz, L. Ramos, S. Aussó, R.M. Morillas, L. Sumoy, M.A. Martinez, C. Tural, Large-scale screening of circulating microRNAs in individuals with HIV-1 mono-infections reveals specific liver damage signatures, Antivir. Res. 155 (2018) 106–114, https://doi.org/10.1016/J.ANTIVIRAL.2018.05.008.

[19] S. Franco, D. Buccione, C. Tural, M.A. Martinez, Circulating microRNA signatures that predict liver fibrosis progression in patients with HIV-1/hepatitis C virus coinfections, AIDS 35 (2021) 1355–1363, https://doi.org/10.1097/QAD.0000000000002895.

[20] S. Franco, R. Pluvinet, J.F. Sanchez-Herrero, L. Sumoy, M.A. Martinez, Rapid and accurate quantification of isomiRs by RT-qPCR, Sci. Rep. 12 (2022) 1–8, https://doi.org/10.1038/s41598-022-22298-7.

[21] I. Balcells, S. Cirera, P.K. Busk, Specific and sensitive quantitative RT-PCR of miRNAs with DNA primers, BMC Biotechnol. 11 (2011), https://doi.org/10.1186/1472-6750-11-70.

[22] C. Sobolewski, N. Calo, D. Portius, M. Foti, MicroRNAs in fatty liver disease, Semin. Liver Dis. 35 (2015) 12–25, https://doi.org/10.1055/S-0034-1397345.

[23] G. Szabo, S. Bala, MicroRNAs in liver disease, Nat. Rev. Gastroenterol. Hepatol. 10 (2013) 542–552, https://doi.org/10.1038/NRGASTRO.2013.87.

[24] M. Gjorgjieva, C. Sobolewski, D. Dolicka, M. Correia De Sousa, M. Foti, MiRNAs and NAFLD: from pathophysiology to therapy, Gut 68 (2019) 2065–2079, https://doi.org/10.1136/gutjnl-2018-318146.

[25] C. Austermann, R. Schierwagen, R. Mohr, E. Anadol, S. Klein, A. Pohlmann, C. Jansen, C.P. Strassburg, C. Schwarze-Zander, C. Boesecke, J.K. Rockstroh, M. Odenthal, J. Trebicka, microRNA-200a: a stage-dependent biomarker and predictor of steatosis and liver cell injury in human immunodeficiency virus patients, Hepatol. Commun. 1 (2017) 36–45, https://doi.org/10.1002/HEP4.1017.

[26] E. Anadol, R. Schierwagen, N. Elfimova, K. Tack, C. Schwarze-Zander, H. Eischeid, A. Noetel, C. Boesecke, C. Jansen, L. Dold, J.C. Wasmuth, C.P. Strassburg, U. Spengler, J.K. Rockstroh, M. Odenthal, J. Trebicka, Circulating microRNAs as a marker for liver injury in human immunodeficiency virus patients, Hepatology 61 (2015) 46–55, https://doi.org/10.1002/HEP.27369.

[27] M. Bulfoni, R. Pravisani, E. Dalla, D. Cesselli, M. Hidaka, C. Di Loreto, S. Eguchi, U. Baccarani, miRNA expression profiles in liver grafts of HCV and HIV/HCV-infected recipients, 6 months after liver transplantation, J. Med. Virol. 93 (2021) 4992–5000, https://doi.org/10.1002/JMV.26999.

[28] Y.L. Liu, G.L. Patman, J.B.S. Leathart, A.C. Piguet, A.D. Burt, J.F. Dufour, C.P. Day, A.K. Daly, H.L. Reeves, Q.M. Anstee, Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma, J. Hepatol. 61 (2014) 75–81, https://doi.org/10.1016/J.JHEP.2014.02.030.

[29] L. Valenti, A. Al-Serri, A.K. Daly, Enrico Galmozzi, R. Rametta, P. Dongiovanni, V. Nobili, E. Mozzi, G. Roviaro, E. Vanni, E. Bugianesi, M. Maggioni, A. LudovicaFracanzani, S. Fargion, C.P. Day, Homozygosity for the patatin-like phospholipase-3/adiponutrin i148m polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease, Hepatology 51 (2010) 1209–1217, https://doi.org/10.1002/hep.23622.

[30] S. Romeo, J. Kozlitina, C. Xing, A. Pertsemlidis, D. Cox, L.A. Pennacchio, E. Boerwinkle, J.C. Cohen, H.H. Hobbs, Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease, Nat. Genet. 40 (2008) 1461–1465, https://doi.org/10.1038/ng.257.

[31] E. Vilar-Gomez, C.J. Pirola, S. Sookoian, L.A. Wilson, T. Liang, N. Chalasani, PNPLA3 rs738409 and risk of fibrosis in NAFLD: exploring mediation pathways through intermediate histological features, Hepatology (2022), https://doi.org/10.1002/HEP.32491.

[32] M. Pujantell, S. Franco, I. Galván-Femenía, R. Badia, M. Castellví, E. Garcia-Vidal, B. Clotet, R. de Cid, C. Tural, M.A. Martínez, E. Riveira-Muñoz, J.A. Esté, E. Ballana, ADAR1 affects HCV infection by modulating innate immune response, Antivir. Res. 156 (2018) 116–127, https://doi.org/10.1016/j.antiviral.2018.05.012.

[33] C. Li, Y. Gao, K. Zhang, J. Chen, S. Han, B. Feng, R. Wang, L. Chen, Multiple roles of MicroRNA-100 in human cancer and its therapeutic potential, Cell. Physiol. Biochem. 37 (2015) 2143–2159, https://doi.org/10.1159/000438572.

[34] P. Starlinger, H. Hackl, D. Pereyra, S. Skalicky, E. Geiger, M. Finsterbusch, D. Tamandl, C. Brostjan, T. Grünberger, M. Hackl, A. Assinger, Predicting postoperative liver dysfunction based on blood-derived MicroRNA signatures, Hepatology 69 (2019) 2636–2651, https://doi.org/10.1002/hep.30572.

[35] A. Satishchandran, A. Ambade, S. Rao, Y.C. Hsueh, A. Iracheta-Vellve, D. Tornai, P. Lowe, B. Gyongyosi, J. Li, D. Catalano, L. Zhong, K. Kodys, J. Xie, S. Bala, G. Gao, G. Szabo, MicroRNA 122, regulated by GRLH2, protects livers of mice and patients from ethanol-induced liver disease, Gastroenterology 154 (2018) 238–252, https://doi.org/10.1053/j.gastro.2017.09.022, e7.

[36] W.C. Tsai, S. Da Hsu, C.S. Hsu, T.C. Lai, S.J. Chen, R. Shen, Y. Huang, H.C. Chen, C.H. Lee, T.F. Tsai, M.T. Hsu, J.C. Wu, H. Da Huang, M.S. Shiao, M. Hsiao, A. P. Tsou, MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis, J. Clin. Invest. 122 (2012) 2884–2897, https://doi.org/10.1172/JCI63455.

[37] X. Meng, K. Liu, Z. Xiang, X. Yu, P. Wang, Q. Ma, MiR-125b-2-3p associates with prognosis of ccRCC through promoting tumor metastasis via targeting EGR1, Am. J. Transl. Res. 12 (2020) 5575–5585.

[38] J. Chang, J.-T. Guo, D. Jiang, H. Guo, J.M. Taylor, T.M. Block, Liver-specific MicroRNA miR-122 enhances the replication of hepatitis C virus in nonhepatic cells, J. Virol. 82 (2008) 8215–8223, https://doi.org/10.1128/jvi.02575-07.

[39] J. Zhou, L. Yu, X. Gao, J. Hu, J. Wang, Z. Dai, J.F. Wang, Z. Zhang, S. Lu, X. Huang, Z. Wang, S. Qiu, X. Wang, G. Yang, H. Sun, Z. Tang, Y. Wu, H. Zhu, J. Fan, Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma, J. Clin. Oncol. 29 (2011) 4781–4788, https://doi.org/10.1200/JCO.2011.38.2697.

[40] R. Villani, F. Di Cosimo, A.D. Romano, M. Sangineto, G. Serviddio, Serum lipid profile in HCV patients treated with direct-acting antivirals: a systematic review and meta-analysis, Sci. Rep. 11 (2021), https://doi.org/10.1038/S41598-021-93251-3.

[41] M. Spaziante, G. Taliani, G. Marchetti, A. Tavelli, M. Lichtner, A. Cingolani, S. Cicalini, E. Biliotti, E. Girardi, A. Antinori, M. Puoti, A.D. Monforte, A. Cozzi-Lepri, Impact of HCV eradication on lipid metabolism in HIV/HCV coinfected patients: data from ICONA and HepaICONA foundation cohort study, Viruses 13 (2021), https://doi.org/10.3390/V13071402.

[42] O. Özdoğan, S. Yaraş, F. Ateş, E. Üçbilek, O. Sezgin, E. Altıntaş, The impact of direct-acting antiviral treatment on lipid metabolism and insulin resistance in chronic hepatitis C patients: temporary? permanent? Turk. J. Gastroenterol. 31 (2020) 384–392, https://doi.org/10.5152/TJG.2020.19273.