

Supplementary Information index

Supplementary Information 1. Extended information processing pipeline.

Supplementary Information 2. Clinical and epidemiological characteristics of HCV+ and HCV+/HIV+ patients according to fibrosis severity.

Supplementary Information 3. Plasma-derived extracellular vesicles characterization

Supplementary Information 4. Novel miRNAs predicted by miRDeep.

Supplementary Information 5. Hierarchical cluster analysis of the SDE miRNAs.

Supplementary Information 6. SDE miRNAs between $F \geq 2$ and $F < 2$ in the CHC cohort and in the HCV+ and HCV+/HIV+ subgroups.

Supplementary Information 7. Venn's diagram for the SDE miRNAs.

Supplementary Information 8. MicroRNA-gene target enrichment analysis.

Supplementary Information 9. Pathway-enrichment analysis target genes of SDE miRNAs.

Supplementary Information 10. Proposed scores to determine significant fibrosis.

Supplementary Information 11. Classification of CHC cases by APRI and FIB-4 scores.

Supplementary Information (SI)

SI1. Extended Information processing pipeline.

Bioinformatics Analysis.

Raw Information were analyzed with a specific bioinformatic pipeline for the identification of known and novel miRNAs. Quality control was performed with FastQC (v.0.11.3); adapter sequences as well as low-quality base calls ($q < 20$) were trimmed with cutadapt (v.1.13) and processed with miRDeep2 (v.0.0.7). This software allows the alignment of the reads to the reference human genome (GRCh38) using the mapper.pl module based on Bowtie1. Only the alignments with zero mismatches in the seed region and those that did not map to more than five different loci in the genome were retained. Quantification of miRNAs was performed with the quantifier.pl module, which determines the expression of the corresponding known miRNAs in two steps. First, predefined mature miRNA sequences were mapped against the reference precursors in miRBase (v21), a public repository miRNA which contains 2888 human miRNAs sequences [1]. Second, sequencing reads were mapped against the precursor sequences. Reads falling into an interval of two nucleotides upstream and five nucleotides downstream of the mature miRNA sequences were determined for quantification.

Statistical analysis

For Information preprocessing and exploratory analysis the edgeR package (v 3.38.4) was used. First, miRNAs with low read counts were filtered out by the *filterByExpr* function, retaining only those miRNAs that have sufficiently large counts according to the minimum expression criterion [2]. The miRNA count matrix was normalized with Trimmed Mean of M-values (TMM) and count per million (CPM) approaches for library size normalization. Then, for exploration analysis, partial least square discriminant analysis (PLS-DA) with the normalized miRNA counts was performed.

For the significant differentially expressed (SDE) miRNA analysis, miRNA expression differences between groups were analyzed using a binomial negative generalized linear model (bnGLM). The miRNAs with a fold change (FC) ≥ 1.5 ($|\log_2\text{FC}| \geq 0.585$) and an adjusted p -value ≤ 0.2 using the false discovery rate (FDR) were considered SDE miRNAs.

miRNA-based target prediction and pathway enrichment analysis

SDE miRNA-target interactions and pathway enrichment analysis of the target genes was performed. Only experimentally validated interactions were considered. CORALIS R package was used for target enrichment analysis. Only target genes with more than 2 interactions with miRNAs were considered candidates for analysis. A corrected p -value for the false discovery rate (FDR) ≤ 0.05 was considered significant. For the identification of biological processes in which the enriched genes are involved, Gene Ontology (GO) Informationbase was used by means of the *enrichGO* function [3, 4].

Diagnostic value evaluation

The diagnostic value to assess significant fibrosis of SDE miRNAs for each comparison was evaluated by the receiver operating characteristic curves (ROC) using pROC (v 1.18.2). An area under the ROC curve (AUROC) between 0.700 and 0.799 was considered acceptable, 0.800 to 0.899 excellent, and above 0.900 outstanding in terms of discriminatory ability. The cutoff value was determined as the one with the maximal value of the sum of the sensitivity and specificity. Moreover, we developed a new score combining SDE miRNAs. To identify the adjusted variables to be included in the new fibrosis severity score, a stepwise forward analysis was performed, and the Akaike information criterion (AIC) was used to determine the best-fitted model. We randomly divided the CHC cohort into a training set ($\approx 80\%$ of cases) and a test set ($\approx 20\%$ of cases) to test the new fibrosis score.

SI2. Clinical and epidemiological characteristics of HCV+ and HCV+/HIV+ patients according to fibrosis severity.

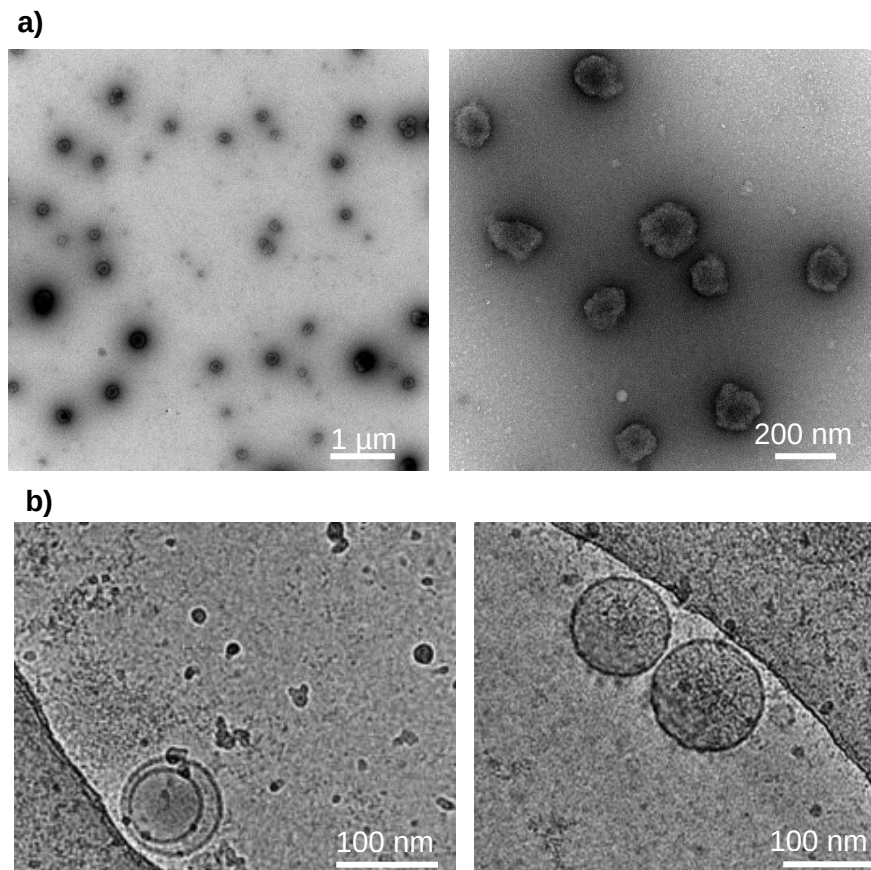
Variables	HCV+		P value
	F<2	F≥2	
<i>n</i>	10	11	
Sex (% female)	5 (50%)	6 (54.5%)	>0.999
Age (years)	49 (37; 57.2)	55 (49; 63)	0.111
BMI (kg/m ²)	26.2 (20.5; 28)	26.1 (22; 27.4)	0.928
Risk of infection			
Parenteral	5	2	>0.999
Sexual	1	1	
Unknown	4	8	
HCV Genotype			
1	9	10	>0.999
2, 3 and 4	1	1	
HCV viral load (UI/ml)	1.7E6 (2.4E5; 7.5E6)	3.5E6 (1.1E6; 9.3E6)	0.360
TC (mg/dL)	189 (178; 211)	193 (153; 220.5)	0.687
GOT (UI/ml)	52 (23.5; 66)	69 (51; 117)	0.0535
GPT (UI/ml)	67 (33; 142)	67 (49; 187)	0.696
GGT (UI/ml)	40 (24; 174)	44 (19; 104)	0.495
ALP (UI/ml)	98 (83.5; 168)	115 (95.2; 142)	0.435
Platelets (10 ³ /ul)	218 (188.2; 237.3)	117.8 (79.1; 216)	0.016*
CD4+ T cel (cel/mm ³)	1114 (691.8; 1410)	713.9 (581.9; 887)	0.112

Variables	HCV+/HIV+		P value
	F<2	F≥2	
<i>n</i>	22	7	
Sex (% female)	14 (63.6 %)	0 (0%)	0.006*
Age (years)	50 (45.7; 53)	48 (45; 53)	0.413
BMI (kg/m ²)	23 (20.6; 26.3)	25.9 (20.8; 27.7)	0.565
Risk of infection			
Parenteral	11	5	0.159
Sexual	8	-	
Unknown	3	2	
Time of HIV infection (months)	262.2 (93.15; 326.4)	345.1 (200; 365.9)	0.146
HCV Genotype			
1	10	7	0.023*
2, 3 and 4	12	0	
HCV viral load (UI/ml)	1.7E6 (3.9E5; 9.3E6)	9.8E6 (4.4E6; 1.4E7)	0.097
TC (mg/dL)	191 (166; 203)	174 (142.5; 187.5)	0.164
GOT (UI/ml)	32 (27; 42)	92 (38.75; 132.5)	0.043*
GPT (UI/ml)	41 (32; 49)	73 (44.5; 150.5)	0.060
GGT (UI/ml)	56 (33; 113)	70 (67; 167)	0.293
ALP (UI/ml)	83 (57.5; 91)	106 (78.5; 145)	0.053
Platelets (10 ³ /ul)	206 (183.5; 234)	162 (137; 216.5)	0.115
CD4+ T cel (cel/mm ³)	685.9 (523.5; 940.5)	866 (530; 1153)	0.253

Values expressed as cases (%) or median (percentile 25; percentile 75). Abbreviations: HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus; BMI: Body Mass Index; TC: total cholesterol; GOT: Glutamic oxaloacetic transaminase; GPT: glutamic pyruvic transaminase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase. * Statistically significant differences between F<2 and F≥2 groups (p < 0.05). Note that F≥2 HCV+ group is conformed by 1 F2, 8 F3 and 2 F4 cases, meanwhile the HCV+/HIV+ by 2 F2, 3 F3 and 2 F4.

SI3. Plasma-derived extracellular vesicles characterization.

Microscopy characterization of plasma-derived extracellular vesicles from HCV+ and HCV+/HIV+ patients. Representative images of the isolated plasma-derived vesicles by **a)** Transmission electron microscopy (TEM) and **b)** Cryoelectron microscopy.



Size range and concentration of plasma-derived extracellular vesicles from enrolled patients. The table details NTA size and concentration median (percentile 25; percentile 75) and *p*-values for the comparison between F<2 and F≥2 results.

Cohort	Fibrosis severity	Size (nm)	<i>P</i> value	Concentration (particles/ml)	<i>P</i> value
CHC	F<2	172.2 (155.7; 186.7)	0.287	5.5E09 (4.2E09; 1E09)	0.897
	F≥2	175.1 (162.5; 192.2)		5.2E09 (4E9; 7.1E09)	
HCV+	F<2	182.2 (161.2; 188.3)	0.820	6.5E09 (4.9E9; 7.9E09)	0.289
	F≥2	176.8 (162.4; 193.0)		5.2E09 (3.9E9; 7.1E09)	
HCV+/HIV+	F<2	168.8 (149.5; 182.4)	0.535	5.4E09 (3.4E9; 6.5E09)	0.428
	F≥2	172.9 (162.2; 189.4)		6.4E09 (4.3E9; 7.7E09)	

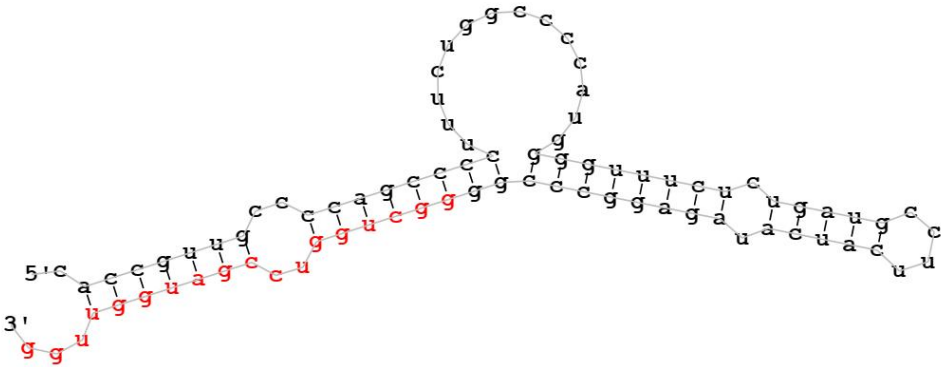
SI4. Novel miRNAs predicted by miRDeep.

Details of the 10 putative *de novo* miRNAs.

Provisional id	miRDeep2 score	Estimated probability that the miRNA candidate is a true positive	Mature read count	Star read count	Significant randfold p-value	Consensus mature sequence
chr3_8792	62.1	13 +/- 14%	123	1	yes	aaagcaggauucagacuacaauau
chr3_7520	60.1	13 +/- 14%	120	7	yes	ucaaggaacuaagaguuuu
chr22_42764	5.5	34 +/- 13%	225	0	yes	ucccuguccuccaggagcuc
chr6_18336	5.2	34 +/- 13%	114	0	yes	ugagguaguagguggugugc
chr7_20006	5.2	34 +/- 13%	1224	0	yes	ggcugguccgaugguugg
chrX_43146	4.9	28 +/- 11%	216	0	yes	caggaggaaggugaggau
chr9_24472	4.9	28 +/- 11%	591	0	yes	ugggugguggugcaugucu
chr8_21271	4.7	28 +/- 11%	593	0	yes	cacagguggugcauggcug
chr8_21793	4.5	28 +/- 11%	286	0	yes	aaggagcucacagucuauug
chr3_10165	4.1	28 +/- 11%	897	1	yes	uguaaacauccuugacu

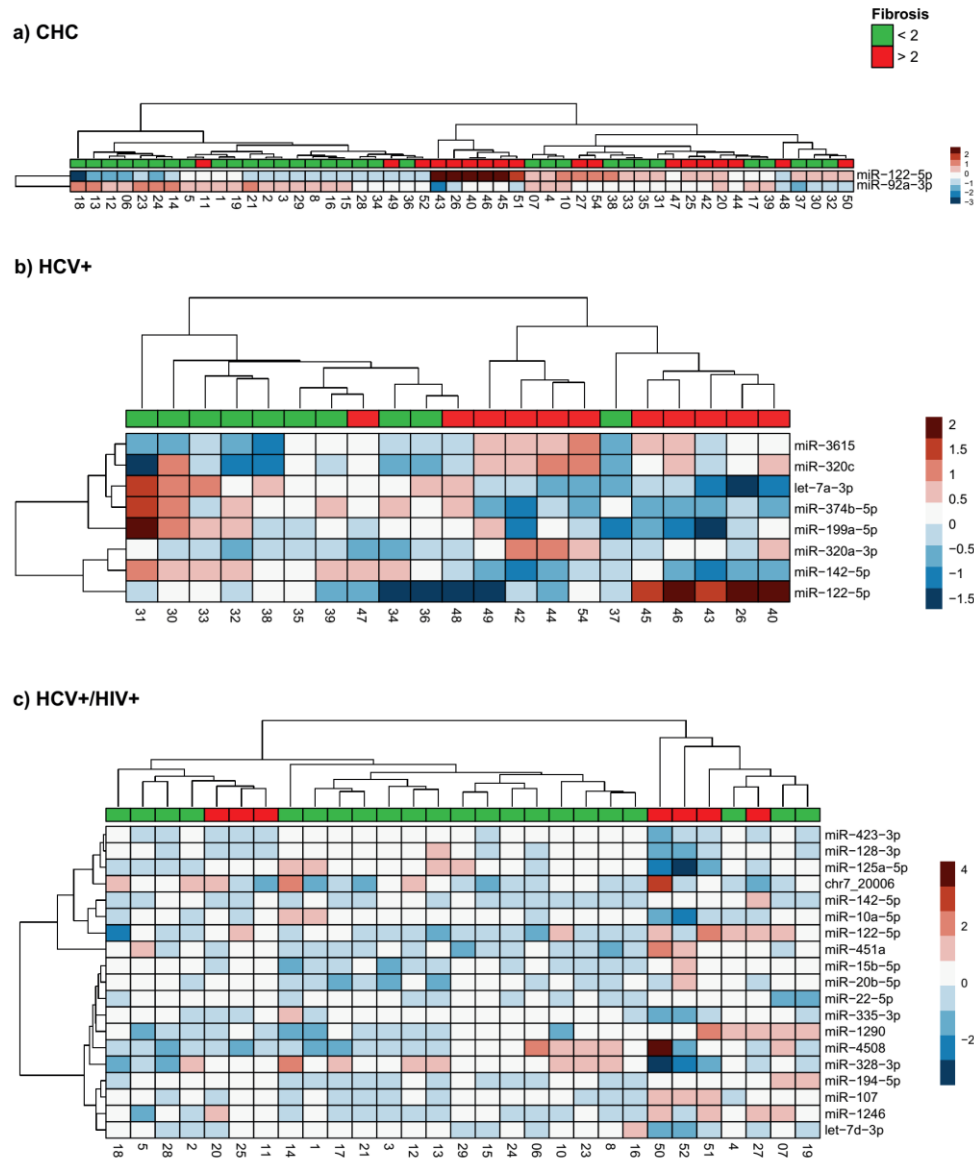
Column 1 represents the identifier generated by mirdeep2 according to the genomic location of the identified *de novo* RNAs; column 2-6 represent parameters estimated by miRDeep2 according to the probability of being a true miRNA, its counts, the RANDFOLD test (calculates the free energy of folding [secondary structure] of the miRNA to determine the possibility of being a miRNA) and its sequence.

Secondary structure of the microRNA *de novo* chr7_20006 determined with mirdeep2.



SI5. Hierarchical cluster analysis of the SDE miRNAs.

Study subjects are represented in columns and colors according to the fibrosis severity group, where green squares represent $F < 2$ and red squares $F \geq 2$ cases. SDE miRNAs are represented in rows. Clustering dendrograms are shown on the left for miRNAs and at the top for samples. The color scale indicates the expression level of SDE miRNAs. The Information was scaled using the *rowMeans()* function, resulting in negative values for all miRNAs with relative expressions lower than the average expression of the matrix. Red color indicates a high expression level and blue a low expression level.



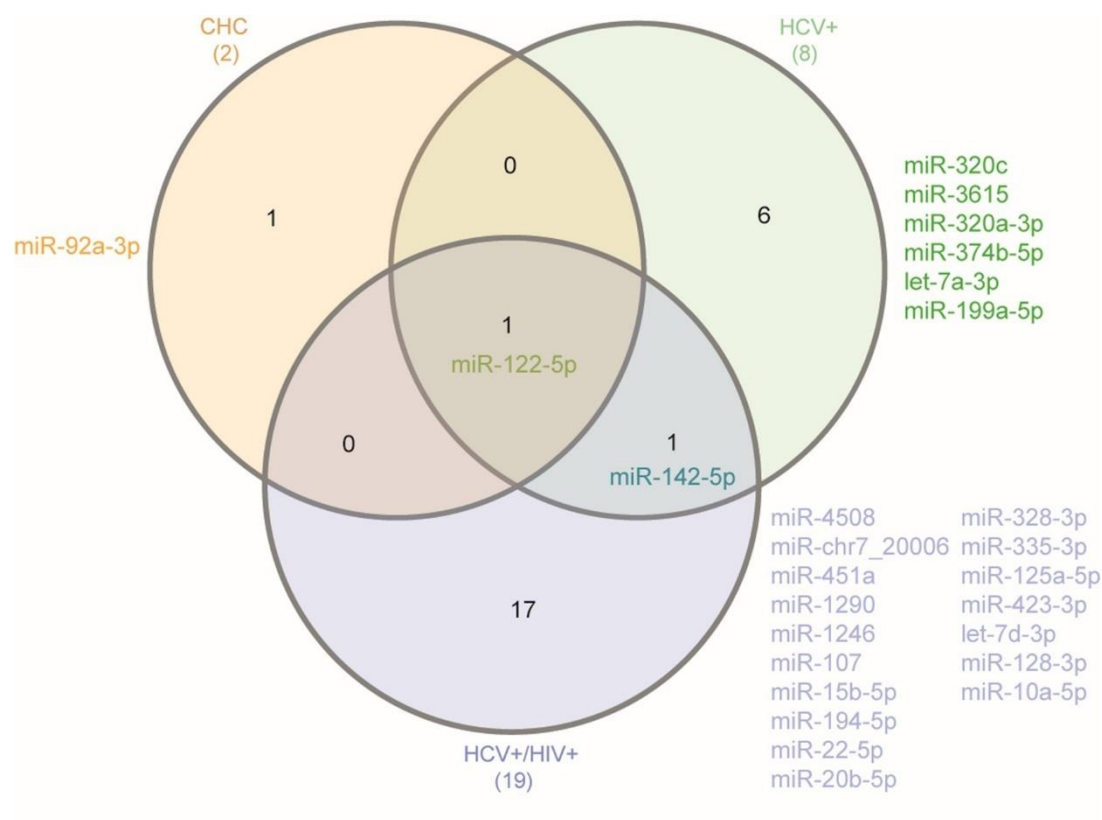
SI6. SDE miRNAs between $F \geq 2$ and $F < 2$ in the CHC cohort and in the HCV+ and HCV+/HIV+ subgroups.

CHC					
Upregulated miRNAs			Downregulated miRNAs		
miRNA	FC	FDR	miRNA	FC	FDR
hsa-miR-122-5p	3.06	<0.001	hsa-miR-92a-3p	-1.5	0.051
HCV+					
Upregulated miRNAs			Downregulated miRNAs		
miRNA	FC	FDR	miRNA	FC	FDR
hsa-miR-122-5p	2.68	0.135	hsa-miR-374b-5p	-2.02	0.088
hsa-miR-320c	2.00	0.098	hsa-let-7a-3p	-1.87	0.088
hsa-miR-3615	1.99	0.012	hsa-miR-199a-5p	-1.67	0.176
hsa-miR-320a-3p	1.56	0.027	hsa-miR-142-5p	-1.59	0.088
HCV+/HIV+					
Upregulated miRNAs			Downregulated miRNAs		
miRNA	FC	FDR	miRNA	FC	FDR
hsa-miR-4508	3.66	0.188	hsa-miR-328-3p	-2.21	0.117
hsa-chr7_20006	2.76	0.143	hsa-miR-335-3p	-1.76	0.117
hsa-miR-122-5p	2.36	0.117	hsa-miR-125a-5p	-1.65	0.191
hsa-miR-451a	2.03	0.071	hsa-miR-423-3p	-1.60	0.021
hsa-miR-1290	2.03	0.138	hsa-let-7d-3p	-1.55	0.136
hsa-miR-1246	1.98	0.078	hsa-miR-128-3p	-1.54	0.071
hsa-miR-107	1.82	0.071	hsa-miR-10a-5p	-1.54	0.117
hsa-miR-15b-5p	1.75	0.117			
hsa-miR-194-5p	1.72	0.071			
hsa-miR-22-5p	1.65	0.128			
hsa-miR-20b-5p	1.60	0.188			
hsa-miR-142-5p	1.60	0.071			

Each column represent: the SDE miRNA identifier (miRBase format), the FC indicating the expression fold change between $F \geq 2$ vs $F < 2$ for each comparison group, and the adjusted p -value (FDR, corrected for multiple testing with the Benjamini– Hochberg procedure).

SI7. Venn’s diagram for the SDE miRNAs.

The Venn diagram displays the number of shared and non-shared significant differentially expressed miRNAs among different groups of patients.



SI8. MicroRNA-gene target enrichment analysis.

Top 25 gene-miRNA interactions according to the FDR obtained in the target-enrichment analysis for the SDE miRNAs in each comparison.

Interactive miRNA-gene networks for the CHC group and the HCV+ and HCV+/HIV+ subgroups are available in the following link:

<https://valvapamela.github.io/MicroRNA-enrichment-analysis/>

Results of the SDE miRNAs' target genes functional analysis.

CHC				
Gene symbol	Function involved	Number of interactions	miRNAs	FDR
CYP7A1	Drug metabolism and cholesterol, steroids, and other lipid synthesis	2	hsa-miR-122-5p / hsa-miR-92a-3p	9.92E+09
GLOD4	Cadherin binding activity	2	hsa-miR-122-5p / hsa-miR-92a-3p	9.92E+09
ADAM10	Protein processing (metalloproteinase)	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0003
DYNC1H1	Intracellular transport (dynein motor)	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0003
USP10	Regulation of ubiquitination and protein recycling	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0003
LPIN1	Lipid metabolism (triglyceride synthesis)	2	hsa-miR-92a-3p / hsa-miR-122-5p	0.0003
MAPRE1	Regulation of microtubule structures/Chromosomal stability	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0003
NCDN	Neuronal signaling (memory and learning)	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0003
SPTLC1	Sphingolipid biosynthesis	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0003
TGFBRAP1	TGF- β signaling transduction	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0003
HLA-E	Antigen presentation (immune response)	2	hsa-miR-92a-3p / hsa-miR-122-5p	0.0004
KIAA0100	Cytoskeleton regulation/Lipid transport/Cancer	2	hsa-miR-92a-3p / hsa-miR-122-5p	0.0005
OPTN	Cellular morphogenesis/Membrane trafficking/Vesicle trafficking/Transcription activation	2	hsa-miR-92a-3p / hsa-miR-122-5p	0.0005
RAD21	Sister chromatid cohesion (DNA repair)	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0005
ANXA11	Vesicle transport/Apoptosis	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0005
ATP7A	Copper transport	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0005
FMN1	Cytoskeleton organization	2	hsa-miR-92a-3p / hsa-miR-122-5p	0.0005

SLC9A1	Proton and sodium exchange (pH homeostasis)/Cell migration/Cell volume	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0005
USP28	DNA damage response/ Cancer	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0005
DENND2C	G protein activation/Cell signaling regulation/Enzymatic activity modulation	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0006
IFITM1	Antiviral response (innate immunity)	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0007
ARL2	Regulation of microtubules and centrosomes/STAT3 regulation	2	hsa-miR-92a-3p / hsa-miR-122-5p	0.0007
TRAM2	Reticulum endoplasmic posttranslational secretory control	2	hsa-miR-122-5p / hsa-miR-92a-3p	0.0008
EFTUD2	RNA splicing	2	hsa-miR-92a-3p / hsa-miR-122-5p	0.0008
DMXL1	Cell signaling regulation/Cell process control/Protein complex interaction and assembly	2	hsa-miR-92a-3p / hsa-miR-122-5p	0.0008

HCV+				
Gene symbol	Function involved	Number of interactions	miRNAs	FDR
NOD2	Innate immune response (pathogen recognition)	2	hsa-miR-122-5p / hsa-miR-320c	0.0135
AIMP1	Angiogenesis control/Inflammation/Wound healing	2	hsa-miR-142-5p / hsa-miR-320c	0.0135
GSK3B	Glucose homeostasis/Energy metabolism/Inflammation/ER stress/Mitochondrial dysfunction/Apoptosis	3	hsa-let-7a-3p / hsa-miR-199a-5p / hsa-miR-374b-5p	0.0135
RAC1	Cell growth/Cytoskeleton reorganization/Protein kinase activation	3	hsa-miR-122-5p / hsa-miR-142-5p / hsa-miR-374b-5p	0.0135
SLC38A2	Amino acid transport/Cellular response to arsenite/RNA splicing	3	hsa-miR-199a-5p / hsa-miR-142-5p / hsa-miR-320c	0.0135
SPTLC1	Sphingolipid biosynthesis	2	hsa-miR-374b-5p / hsa-miR-122-5p	0.0162
APOBEC3A	RNA/DNA editing/Antiviral defense	2	hsa-let-7a-3p / hsa-miR-122-5p	0.0193
NIPA2	Magnesium ion transport	2	hsa-miR-3615 / hsa-miR-122-5p	0.0193
TTC8	Cilium formation and maintenance	2	hsa-miR-374b-5p / hsa-miR-142-5p	0.0193
ACSM2A	Fatty acid metabolism	2	hsa-miR-3615 / hsa-miR-374b-5p	0.0211
SMAD3	TGF- β signaling transduction	2	hsa-miR-142-5p / hsa-miR-199a-5p	0.0211
SRSF7	RNA splicing regulation	3	hsa-miR-374b-5p / hsa-miR-142-5p / hsa-miR-320c	0.0211
EGLN3	Hypoxia response (HIF hydroxylation)/DNA damage/Apoptosis	2	hsa-miR-122-5p / hsa-miR-142-5p	0.0217
EIF2A	Translation initiation (protein synthesis)	2	hsa-miR-142-5p / hsa-miR-320c	0.0217

GTF2B	Gene transcription (general transcription factor)	2	hsa-miR-122-5p / hsa-miR-142-5p	0.0217
MCAM	Cell adhesion (tumor invasion and angiogenesis)	2	hsa-miR-122-5p / hsa-miR-142-5p	0.0217
SIRT1	Cell cycle/DNA damage response/Metabolism/Apoptosis/Autophagy/Epigenetic gene silencing	2	hsa-miR-199a-5p / hsa-miR-142-5p	0.0217
SPAG9	c-Jun N-terminal kinase (JNK) signaling/Microtubule membrane transport/Cancer	2	hsa-miR-122-5p / hsa-let-7a-3p	0.0217
TGFB2	Regulation of cell proliferation and differentiation/Immune response	2	hsa-miR-199a-5p / hsa-miR-142-5p	0.0217
LUZP2	Transcription factor dimerization	2	hsa-miR-374b-5p / hsa-miR-142-5p	0.0217
ATP13A3	Cation transport	2	hsa-miR-122-5p / hsa-miR-142-5p	0.0223
CREBRF	Stress response/Transcription regulation/Transport	3	hsa-let-7a-3p / hsa-miR-142-5p / hsa-miR-320c	0.0223
EZH2	Gene transcription regulation	2	hsa-miR-199a-5p / hsa-miR-320c	0.0223
PHKA1	Glycogen metabolism regulation	2	hsa-miR-122-5p / hsa-let-7a-3p	0.0223
SOCS1	Negative regulation of cytokine signaling (JAK/STAT inhibition)/Immune response/Cell growth	2	hsa-miR-122-5p / hsa-miR-142-5p	0.0223

HCV+/HIV+				
Gene symbol	Function involved	Number of interactions	miRNAs	FDR
JAK1	Kinase/Transferase/IFN- α / β / γ signaling (JAK/STAT)/Inflammation/Cancer	4	hsa-miR-128-3p / hsa-miR-15b-5p / hsa-miR-107 / hsa-miR-20b-5p	0.0036
USP48	Regulation of ubiquitination and protein stability	5	hsa-miR-142-5p / hsa-miR-15b-5p / hsa-miR-1290 / hsa-miR-1246 / hsa-miR-20b-5p	0.0075
CDY2B	Translocase/Chromatin regulation in spermatogenesis	2	hsa-miR-128-3p / hsa-miR-122-5p	0.0090
NOP16	Cancer	2	hsa-miR-125a-5p / hsa-miR-10a-5p	0.0090
SRSF1	RNA splicing regulation	6	hsa-miR-10a-5p / hsa-miR-423-3p / hsa-miR-335-3p / hsa-miR-128-3p / hsa-miR-107 / hsa-miR-15b-5p	0.0090
LIN28A	Regulation of pre-let-7 miRNA maturation/Post-transcriptional gene regulation	3	hsa-miR-125a-5p / hsa-miR-128-3p / hsa-miR-107	0.0090
A2M	Protease inhibition/Inflammatory response	2	hsa-miR-128-3p / hsa-miR-122-5p	0.0103
AJUBA	Cytoskeleton organization/Cell adhesion/Differentiation, proliferation, and migration/Transcription regulation	2	hsa-miR-128-3p / hsa-miR-10a-5p	0.0103
ATP13A3	Translocase/Cation transport	4	hsa-miR-122-5p / hsa-miR-107 / hsa-miR-142-5p / hsa-miR-15b-5p	0.0103
BAX	Apoptosis	3	hsa-miR-122-5p / hsa-miR-128-3p / hsa-miR-15b-5p	0.0103
KIAA0895	Unknown	4	hsa-miR-15b-5p / hsa-miR-1290 / hsa-miR-1246 / hsa-miR-142-5p	0.0103

MCPH1	Cell cycle regulation/Chromosome condensation/DNA damage-induced cellular responses/Neurogenesis	2	hsa-miR-15b-5p / hsa-miR-10a-5p	0.0103
RELN	Extracellular matrix serine protease/Neuron stratification/Neuronal migration	2	hsa-miR-128-3p / hsa-miR-10a-5p	0.0103
ZNF502	Transcriptional regulation	3	hsa-miR-10a-5p / hsa-miR-1246 / hsa-miR-1290	0.0104
MAP3K7	Serine/threonine protein kinase/TGF- β and BMP-induced signaling/Transcription regulation/Apoptosis	4	hsa-miR-10a-5p / hsa-miR-107 / hsa-miR-15b-5p / hsa-miR-335-3p	0.0114
SNTB2	Subcellular organization of membrane proteins/Actin binding/Regulation of secretory granules	5	hsa-miR-10a-5p / hsa-miR-107 / hsa-miR-15b-5p / hsa-miR-20b-5p / hsa-miR-122-5p	0.0114
E2F7	Transcription factor/Cell cycle regulation	4	hsa-miR-125a-5p / hsa-miR-128-3p / hsa-miR-10a-5p / hsa-miR-15b-5p	0.0118
RBM17	RNA splicing regulation	2	hsa-miR-125a-5p / hsa-miR-10a-5p	0.0127
SF3B4	RNA splicing/Spliceosome assembly	2	hsa-miR-423-3p / hsa-miR-10a-5p	0.0127
SLC2A3	Glucose transport (GLUT3)	4	hsa-miR-10a-5p / hsa-miR-122-5p / hsa-miR-15b-5p / hsa-miR-107	0.0127
UBE2L3	Protein ubiquitination (E2 ubiquitin-conjugating enzyme)	2	hsa-miR-122-5p / hsa-miR-125a-5p	0.0127
APRT	Purine metabolism	2	hsa-miR-15b-5p / hsa-miR-10a-5p	0.0148
BPGM	Regulation of hemoglobin's oxygen affinity	2	hsa-miR-128-3p / hsa-miR-122-5p	0.0148
DDX5	RNA unwinding (helicase)/Transcription regulation	3	hsa-miR-15b-5p / hsa-miR-142-5p / hsa-miR-20b-5p	0.0148
DDX56	Transcription regulation/Ribosome biogenesis/Nucleolus organization	2	hsa-miR-423-3p / hsa-miR-328-3p	0.0148

Each column represent: the target gene symbol (Gene symbol), function involved, the number of SDE miRNAs that modulate each enriched gene (Number of interactions), the miRNA identifier in miRBase format and the adjusted *p*-value (FDR, corrected *p*-value for multiple testing performed with the Benjamini–Hochberg procedure).

SI9. Pathway-enrichment analysis of the target genes.

Results of the pathway-enrichment analysis for the SDE miRNAs obtained by GO BP (biological processes).

CHC		
ID	Description	FDR
GO:0051495	positive regulation of cytoskeleton organization	0.0693
GO:0032273	positive regulation of protein polymerization	0.0693
GO:0044089	positive regulation of cellular component biogenesis	0.0797

HCV+		
ID	Description	FDR
GO:0010934	macrophage cytokine production	0.0118
GO:0010935	regulation of macrophage cytokine production	0.0118
GO:0045785	positive regulation of cell adhesion	0.0118
GO:0010508	positive regulation of autophagy	0.0118
GO:0061082	myeloid leukocyte cytokine production	0.0118
GO:0001954	positive regulation of cell-matrix adhesion	0.0118
GO:0010718	positive regulation of epithelial to mesenchymal transition	0.0118
GO:0001837	epithelial to mesenchymal transition	0.0124
GO:2000109	regulation of macrophage apoptotic process	0.0150
GO:0030308	negative regulation of cell growth	0.0150
GO:0010936	negative regulation of macrophage cytokine production	0.0157
GO:0016239	positive regulation of macroautophagy	0.0157
GO:0071888	macrophage apoptotic process	0.0157
GO:0050678	regulation of epithelial cell proliferation	0.01567
GO:2000736	regulation of stem cell differentiation	0.0157
GO:0045860	positive regulation of protein kinase activity	0.0157
GO:0010921	regulation of phosphatase activity	0.0157
GO:0001558	regulation of cell growth	0.0157
GO:0045936	negative regulation of phosphate metabolic process	0.0172
GO:0010563	negative regulation of phosphorus metabolic process	0.0172
GO:1900034	regulation of cellular response to heat	0.0172
GO:0051492	regulation of stress fiber assembly	0.0172
GO:0045444	fat cell differentiation	0.0172
GO:0045926	negative regulation of growth	0.0172
GO:0048762	mesenchymal cell differentiation	0.0172

HCV+/HIV+		
ID	Description	FDR
GO:2000637	positive regulation of miRNA-mediated gene silencing	0.0011
GO:0060148	positive regulation of post-transcriptional gene silencing	0.0011
GO:1900370	positive regulation of post-transcriptional gene silencing by RNA	0.0011

GO:0060964	regulation of miRNA-mediated gene silencing	0.0039
GO:1900368	regulation of post-transcriptional gene silencing by RNA	0.0039
GO:0060147	regulation of post-transcriptional gene silencing	0.0039
GO:0060966	regulation of gene silencing by RNA	0.0039
GO:1903800	positive regulation of miRNA maturation	0.0107
GO:0000380	alternative mRNA splicing, via spliceosome	0.0130
GO:0021987	cerebral cortex development	0.0346
GO:1903798	regulation of miRNA maturation	0.0380
GO:0070920	regulation of production of small RNA involved in gene silencing by RNA	0.0396
GO:0000377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	0.0396
GO:0000398	mRNA splicing, via spliceosome	0.0396
GO:0000375	RNA splicing, via transesterification reactions	0.0396

Each column represent: the pathway ID, the description of the pathway and the adjusted *p*-value (FDR, corrected *p*-value for multiple testing performed with the Benjamini–Hochberg procedure). For CHC table shows pathways with FDR <0.1. For HCV+ subgroup, table shows the top 25 pathways with FDR <0.05. For HCV+/HIV+ subgroup, table shows all pathways with FDR<0.5.

SI10. Proposed score to determine significant fibrosis.

Considering that the diagnostic value of the SDE miRNAs in the CHC group was only acceptable (hsa-miR-92a-3p: AUROC 0.767, Sp 68.8, Se 88.9; hsa-miR-122-5p: AUROC 0.746, Sp 65.6, Se 77.8), a multiple logistic regression analysis (binomial) was performed to develop a new score including both miRNAs as predictor of significant fibrosis. The dependent variable was the METAVIR fibrosis score (dichotomous: $F \geq 2$ and $F < 2$), while the explanatory variables included the normalized counts of the SDE miRNAs. To determine the variables to be included in the new fibrosis severity score, a stepwise forward regression analysis was performed, and the Akaike information criterion (AIC) was used to determine the best fitted model.

Although the cohorts have low number of cases, the Information set of CHC individuals was randomly divided into a training set (approximately 80% of the cases) and a test set (approximately 20% of the cases) to develop and test the fibrosis severity new score. The new score was created using the Information set from the training set. The coefficients (β) obtained from the regression analysis were used to estimate the probability using the following equation:

$$p = \frac{e^{(\beta_0 + \beta_1 X_1 + \beta_2 X_2)}}{(1 + e^{(\beta_0 + \beta_1 X_1 + \beta_2 X_2)})}$$

The diagnostic value of the proposed new score was evaluated using receiver operating characteristic curves (AUROC) with pROC (v 1.18.2). The cutoff value for diagnosis was determined as the maximum value of the sum of sensitivity and specificity. The detailed results are presented in the following table.

Proposed new score						
Selected variables	β coefficients	AUROC	Cut off	95%CI	Sp (%)	Se (%)
X1: has-miR-122-5p X2: hsa-miR-92a-3p	β_0 1.077 β_1 1.538e-05 β_2 -2.923e-04	0.858	0.235	0.71-0.95	74.1	100

Each column represents: selected variables and their coefficients (β) of the proposed new score, the area under the ROC curve, the cutoff value for the proposed score, the 95% confidence interval, specificity, and sensitivity.

SI11. Classification of CHC cases by APRI and FIB-4 scores.

To compare the proposed model with other indexes widely used in clinical hepatology, APRI and FIB4 were calculated for each patient of the training set according to Information availability. The diagnostic value to assess liver fibrosis severity was evaluated. Both indexes displayed excellent diagnostic performance (AUROC APRI=0.815 and AUROC FIB4=0.833). Therefore, considering the cut-off values of APRI (< 0.5 no significant fibrosis, 0.5–1.5 indeterminate, > 1.5 for F ≥ 2) and FIB-4 (< 1.45 no significant fibrosis, 1.45–3.25 indeterminate, > 3.25 for F3-4), cases were classified. The table displays true positive, true negative, false positive and false negative values for both indexes and for the proposed new score.

	APRI	FIB-4	CHC proposed new score
TP	7	6	13
TN	12	14	20
FP	0	0	7
FN	2	2	0
Indeterminate	15	14	-
na	4	4	-

TP: true positive; TN: true negative; FP: false positive; FN: false negative; na: not available.

Those indeterminate cases by APRI and FIB-4 were evaluated by the proposed new score to determine whether it could correctly classify them. The following table describes the results.

	CHC New score			
	TP	TN	FP	FN
Indeterminate APRI (n=15)	2	9	4	0
Indeterminate FIB-4 (n=14)	3	10	1	0

TP: true positive; TN: true negative; FP: false positive; FN: false negative.

References

- [1] Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res.* 2014;42:D68-7310.1093/nar/gkt1181.
- [2] Chen Y, Lun AT, Smyth GK. From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using Rsubread and the edgeR quasi-likelihood pipeline. *F1000Res.* 2016;5:143810.12688/f1000research.8987.2.
- [3] Valle-Millares D, Briz V, Fernández-Rodríguez A. CORALIS, an R package for ncRNA-target enrichment analysis based on experimentally validated interactions. *Research Square*2022;10.21203/rs.3.rs-2199419/v1.
- [4] Valle-Millares D, Brochado-Kith O, Martin-Carbonero L, Dominguez-Dominguez L, Ryan P, De Los Santos I, et al. Different HCV Exposure Drives Specific miRNA Profile in PBMCs of HIV Patients. *Biomedicines.* 2021;910.3390/biomedicines9111627.