Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 13, Issue 3, 2023, 213
https://doi.org/10.33263/BRIAC133.213

Epigenetic Modulation in Viral Hepatitis C Infected Patients after Successful Therapy

Teodora Isac [1], Gabriela Droc [2,3], Artsiom Klimko [4], Mihail Cotorogea-Simion [2], Maria Dobre [5], Elena Milanesi [5], Natalia Cucu [6], Andra-Elena Balcangiu-Stroescu [7], Laura Iliescu [1,†], Sebastian Isac [2,8,†]

1 Department of Internal Medicine II, Faculty of Medicine, 'Carol Davila' University of Medicine and Pharmacy, 020021 Bucharest, Romania; teodora.isac@drd.umfcd.ro (T.I.), laura.iliescu@umfcd.ro (L.I.)
2 Department of Anesthesiology and Intensive Care I, 'Fundeni' Clinical Institute, 022328, Bucharest, Romania; gabriela.droc@umfcd.ro (G.D.), mihail.cotorogea@gmail.com (M.C.S.), sebastian.isac@umfcd.ro (S.I.)
3 Department of Anesthesiology and Intensive Care I, Faculty of Medicine, University of Medicine and Pharmacy, Carol Davila, 020021, Bucharest, Romania; gabriela.droc@umfcd.ro (G.D.)
4 Laboratory of Molecular Neuro-Oncology, Department of Neurology, University Hospital Zurich, 8091, Zürich, Switzerland; artsiom.klimko@usz.ch (A.K.)
5 Department of Cellular and Molecular Medicine ""Victor Babes"" National Institute of Pathology, 050096, Bucharest, Romania; maria.dobre@ivb.ro (M.D.), elena.milanesi@ivb.ro (E.M.)
6 Association of Epigenetics and Metabolomics, Bucharest, Romania; nataliacucu@gmail.com (N.C.)
7 Department of Physiology, Faculty of Dentistry, Carol Davila University of Medicine and Pharmacy, 020021, Bucharest, Romania; stroescu_andra@yahoo.ro (A.E.B.S.)
8 Department of Physiology, Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, 020021, Bucharest, Romania; sebastian.isac@umfcd.ro (S.I.)
* Correspondence: gabriela.droc@umfcd.ro (G.D.);
† The authors have the same contribution

Scopus Author ID 6507520655
Received: 28.03.2022; Accepted: 16.04.2022; Published: 5.06.2022

Abstract: Nowadays, hepatitis C-virus (HCV) represents a challenging liver condition for infected patients and health care systems worldwide. Thus, identifying new biomarkers for early diagnosis and therapy response prediction should be prioritized. Our study aims to identify new epigenetic markers in patients with HCV infection before and after successful direct-acting agents therapy (DAA) and correlate them with standard diagnostic tools. We analyzed blood samples from 12 healthy volunteers and 22 HCV infected patients, before and 3 months after DAA, by assessing: liver transaminases, alpha-fetoprotein, cryoglobulinemia, miR-7-1-3p, miR-21-3p, miR122-5p, miR-885-5p, miR-16-5p, and liver fibrosis (FibroScan®). The study groups were Ctrl (Control group), HCV (HCV infected patients before therapy), and SVR (sustained viral response group). miR-7-1-3p was up-regulated in SVR compared to HCV. No difference was observed between Ctrl and HCV. MiR-21-3p was up-regulated in SVR compared to Ctrl. MiR-122-5p was up-regulated in both, HCV and SVR groups, while miR-885-5p was up-regulated in HCV and down-regulated in SVR group. Moreover, miR-122-5p and miR-885-5p were correlated with liver cytolysis. Our results revealed an innovative panel of epigenetic biomarkers in the early stage of HCV infection and its variation after successful DAA therapy.

Keywords: hepatitis C; microRNA; epigenetics; direct-acting agents; sustained viral response.

1. Introduction

According to the most recent estimates by the World Health Organization (WHO), there are over 58 million people who are seropositive for Hepatitis C virus (HCV) [1]. HCV has a

strong predilection for progression to chronic infection, with up to 85% of acute infections becoming chronic [2]. Patients with chronic HCV suffer from a high symptom burden, with late disease sequelae including cirrhosis, hepatic decompensation, and in a third of cases, hepatocellular carcinoma (HCC) [3].

The indolent but aggressive disease course leads to an age-adjusted mortality rate higher than that of the human immunodeficiency virus [4]. Protracted latency of the early stages of HCV infection hinders effective detection, with empiric screening necessary to identify infected patients. Moreover, the evolution of hepatocellular carcinoma is unpredictable and serological markers are still inconclusive in the early stages [5].

New diagnostic strategies involving modulatory epigenetic biomarkers in various hepatic and non-hepatic pathologies represent promising tools in the therapeutic management of patients [6–10]. Emerging research identifies small noncoding ribonucleic acid (RNA) molecules, namely microRNAs (microRNAs or miRs), as promising therapeutic targets and biomarkers with diagnostic and prognostic potential [11–14]. In addition to stabilizing the viral genome during translation and replication, miRNAs modulate the innate and adaptive immune system via post-transcriptional regulation [15,16].

Chronic HCV infection can lead to a dysregulated profile of endogenous microRNAs, contributing to inflammation, fibrosis, and disease progression [17]. Therefore, this study aims to validate a new panel of microRNAs, as epigenetic markers, in the early stage of HCV infection after normalization to healthy volunteers, to assess the modulatory potential of these markers secondary to direct-acting agents (DAA) therapy in HCV infected patients with the sustained viral response (SVR) after therapy and to correlate these values with biochemical and imagistic data to appraise their prognostic utility.

2. Materials and Methods

2.1. Study design. Inclusion/exclusion criteria.

This study is a monocentric, double-blinded, prospective clinical trial that focused on comparing new epigenetic markers in HCV-infected patients to healthy volunteers and their changes secondary to DAA therapy. Secondary, we analyzed potential correlations of these markers with some biochemical and imagistic data such as AST, ALT, alpha-fetoprotein, cryoglobulinemia, and FibroScan® score.

All clinical procedures were carried out following approval from the local Ethics Committee of Fundeni Clinical Institute (Romania) (no. 48358/01.10.2019) for clinical trials following the European Communities Council Directive 2001/20/EC and respecting personal data privacy (European Directive 95/46/EC). Informed consent was obtained from all subjects included in the study.

We included patients with detectable HCV infection over six months, life expectancy over 12 months, no decompensated cirrhosis, and healthy volunteers. The patients with structural renal disease, decompensated heart failure, concurrent alcohol and drug use, an extrahepatic manifestation of HCV infection, HBV co-infection, and HIV co-infection were excluded. A convenience sample size was used.

In healthy volunteers, we assessed plasmatic AST, ALT, alpha-fetoprotein, cryoglobulinemia, miR 7-1-3p, miR-21-3p, miR122-5p, miR-885-5p, miR-16-5p and the liver fibrosis by FibroScan®. In HCV patients, we assessed the above-mentioned parameters at two different time points: at the therapy induction and 3 months later, when the patients reached

SVR. The AST and ALT were assessed spectrophotometrically and expressed in U/l. The plasmatic AFP (ng/ml) and the presence of mixed cryoglobulinemia were determined on an Advia Centaur XPT Reader (Siemens, Erlangen- Germany).

For miR species, total RNA was isolated from 200µl of plasma with the miRNeasy Serum/Plasma kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and was eluted in 20 µl RNase free water. Three µl of RNA were reverse transcribed in a total volume of 10 µl using miRCURY LNA RT kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. For each sample, six RT-qPCR with 1 µl of cDNA per reaction were performed using the miRCURY LNA SYBR Green PCR kit, and the following miRCURY miRNA Assays (Qiagen, Hilden, Germany): miR-7-1-3p (YP00205888), miR-21-3p (YP00204302), miR122-5p (YP00205664), miR-885-5p(YP00204437) and miR-16-5p (YP00205702) were expressed. The reactions were performed using the 7500 Fast machines (Applied Biosystems, Foster City, CA, USA). The Ct values were normalized according to the delta CT method on the miR-16-5p, as suggested by Qiagen, and after verifying its stability using the RefFinder algorithm [18]. The miR levels were expressed as fold-change to the control group for comparative and as $2^{-\Delta\Delta CT}$ for the correlative analysis.

The non-invasive measurement of liver fibrosis was performed using Fibroscan® technology, Model 530 compact (Echosens, France). According to the literature, the results were expressed automatically in kPa and staged in 4 degrees, with 0 representing no fibrosis and 4 representing major fibrosis.

The therapy consisted of 25 mg ombitasvir, 150 mg paritaprevir, 100 mg ritonavir (AbbVie Deutschland G.m.b.H and Co. KG, Germany), and 500 mg dasabuvir (AbbVie Deutschland G.m.b.H and Co. KG, Germany) per day for three months following international guidelines [19,20].

2.2. Statistical analysis.

The miRNA levels were not normally distributed (Shapiro–Wilk test < 0.05), thus, nonparametric tests were applied. Mann Whitney test was used to compare the miRNA levels between patients and controls. Related samples Wilcoxon signed-rank test was used to assess the differences between patients before and after treatment. The differences in miRNA expression among the groups were considered significant when $p<0.05$. Differences between controls and patients in terms of biochemical and socio-demographic continuous variables were tested with the t-test and, for discrete variables, with the chi-squared test.

Correlations were assessed through Spearman correlation analysis. We considered a clinically relevant correlation if the correlation coefficient was greater than 0.5. Statistical analysis was performed with the software IBM SPSS Statistics (NY, USA), and the graphs were realized using GraphPad Prism 8.4.3 (California, USA).

3. Results and Discussion

3.1. Demographic data.

The 74 patients were initially enrolled between February 2020 and December 2020. Of the enrolled patients, 3 refused to participate, and 28 patients presented at least one exclusion criteria. Of the remaining 43 patients, 9 didn't exhibit a sustained viral response to therapy. Of

the remaining 34 patients, 12 were healthy volunteers, and 22 were enrolled in the study group. The data are presented in Fig.1.

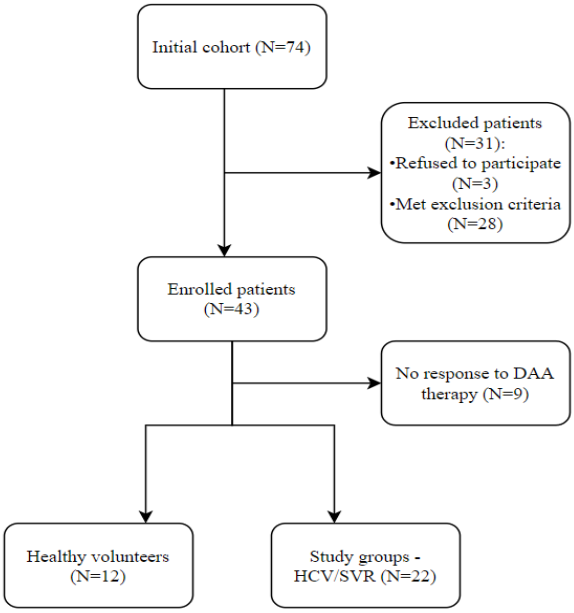

Figure 1. Flow chart representing the patients' selection for the study.

Demographic data, such as age, gender, and smoking status, are summarized in Table 1.

Table 1. Demographic data among study groups.

Parameters	Control group (n=12)	Study group (n=22)	P-value
Mean age (years)	56.3±1.34	62.2±1.98	NS
Gender (F/M %)	59.45/40.54	65.42/34.58	NS
Smoking (%)	13.51	11.42	NS

3.2. The comparative results of miR panel among groups.

The fold-change variations among groups are displayed in Fig.1.

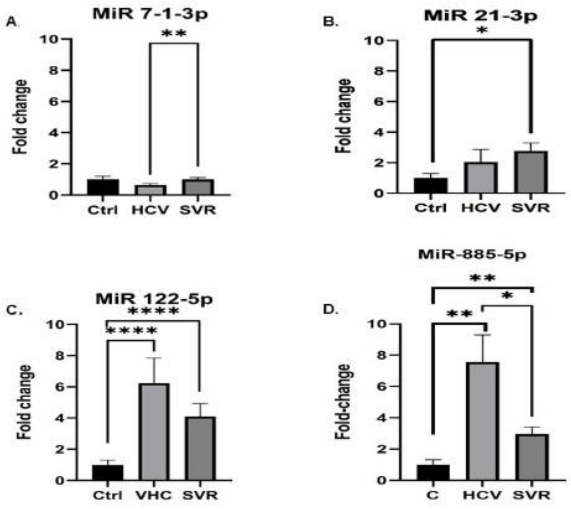

Figure 1. Epigenetic biomarkers expression among groups. (A) MiR 7-1-3p, (B) MiR 21-3p, (C) MiR 122-5p and (D) MiR 885-5p assessed in healthy volunteers (Ctrl), Hepatitis C virus-infected patients with detectable viral load (HCV) and hepatitis C virus-infected patients 12 weeks after sustained viral response (SVR). Bars are mean ± SEM. *P=0.05-0.01, **P=0.01-0.001, ***P=0.001-0.0001, and **** P<0.0001.

No difference was observed between the Ctrl group and the HCV group regarding miR-7-1-3p expression (1±0.2 vs. 0.65±0.08, p=NS). We observed, however, a significant upregulation of miR 7-1-3p in SVR group compared to HCV group: 1.03±0.11 vs. 0.65±0.08 (p<0.006) (Fig.1A). MiR-21-3p was up-regulated only in SVR group: 2.76±0.52 vs. 1±0.29, p=0.17 (Fig.1B). MiR-122 was up-regulated in HCV and SVR groups when compared to the Ctrl group, respectively: 6.24±1.5 vs. 1±0.3 (p<0.001) and 3.96±0.72 vs. 1±0.3 (p<0.001) (Fig.1C).

MiR-885-5p was up-regulated in HCV group when compared to Ctrl group: 7.57±1.7 vs. 1±0.31, p<0.001 and down-regulated in SVR group when compared to HCV group: 2.96±0.44 vs. 7.57±1.7, p=0.007. Furthermore, we observed an up-regulation of miR 885-5p in SVR group when compared to the Ctrl group: 2.96±0.44 vs. 1±0.31, p=0.001 (Fig.1D)

3.3. The correlations between miR panel and biochemical data.

Regarding the correlation analysis of the epigenetic markers and the standard biochemical and sonographic data revealed by FibroScan®, in HCV infected patients, we observed a positive correlation between miR 122-5p and AST levels (r=0.7, p<0.001), miR 122-5p and ALT levels (r=0.77, p<0.001) and miR 885-5p and AST levels (r=0.68, p=0.002) (Fig. 2A-C).

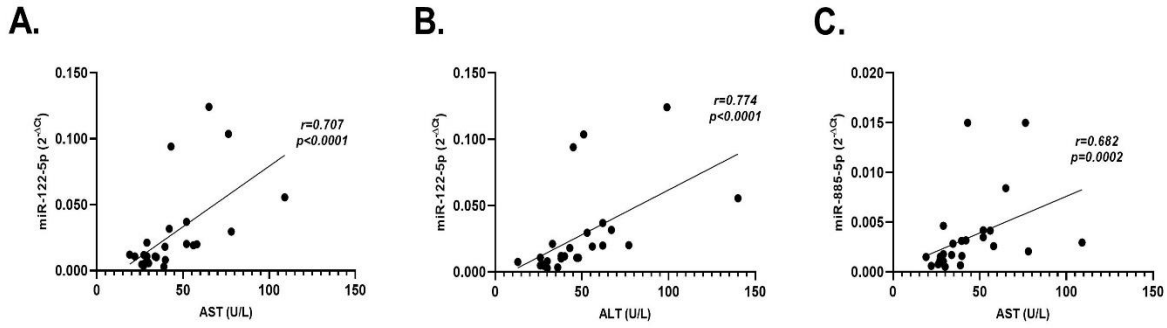

Figure 2. Correlations between serum miR-122-5p and (A) aspartate aminotransferase (AST), (B) alanin aminotransferase (ALT), and between (C) miR-885-5p and aspartate aminotransferase (AST) in the HCV infected patients. r represents the squared correlation coefficient.

3.4. Discussion.

Mature miRs are approximately 20-24 nucleotide-long single-stranded stable RNA molecules which have the potential to revolutionize clinical hepatology. In the present study, we examined the expression of four epigenetic markers (miR-7-1-3p, miR-122-5p, miR-21-3p, and miR-885-5p) in patients with HCV infection to correlate them to clinical parameters and to respond to direct-acting antiviral (DAA) treatment. Our cohort of patients included patients with newly diagnosed HCV infection who were not previously treated and who achieved SVR with DAA therapy – therefore, we believe this represents a valid model to study HCV infection response to DAA therapy.

Mir-7-1 is one of the three precursors of miR-7. One of the main roles of miR-7 is to modulate the metabolic activity of the liver, especially sterol metabolism [21]. Even if clinical signs of any metabolic disturbances are lacking in the acute phase of HCV, the modulatory effect of miR-7 on the liver X receptor signaling could gain significance in the later phases, highlighting the potential impact of nutrigenomics in viral hepatitis. Thus, like other metabolic disturbances (i.e., diabetes mellitus), further precision medicine concepts based on nutrition

and lifestyle could be advocated for a better general outcome in this pathology [22]. Moreover, Vespasiani-Gentilucci *et al*. highlighted that HCV infection could be responsible for viral steatosis, insulin resistance, and diabetes mellitus [23].

Additionally, MiR-7 acts as a tumor suppressor and hepatitis regulator [24]. We found that miR-7-1 expression was decreased in the acute phase of the infection but not during SVR, suggesting the presence of an exacerbated cell growth and proliferative process during the replicative phase in patients with HCV infection in accordance with [24]. This neoplastic process could be epigenetically controlled. Additionally, the downregulation of mi-R 7-1 in HCV-infected patients before DAA induction found in our study could impact the liver steatosis in the acute phase of the disease [25]. Further studies are needed to confirm the linkage between the concomitant liver metabolic changes and the neoplastic process in the acute phase of HCV infection.

The mechanism of action of miR-21 implies a positive correlation with fibrosis staging, viral load, and serum transaminases in chronic HCV infection [25,26]. In our study, miR-21 levels were elevated in acute and SVR patient cohorts, although it was considerably higher in the latter. Mechanistically, miR-21 upregulation occurs in response to infection and during the proliferative phase of hepatic regeneration [27]. It is unclear if miR-21 is a causative agent of fibrosis or if the increases observed in the late stages of chronic HCV infection are surrogates for increased hepatic stroma cell proliferation. Conversely, we found no correlation between miR-21 levels and serum transaminases. In chronic hepatitis B infection, some studies report both elevated and diminished levels of miR-21 in chronic hepatitis without a clear correlation with transaminases [28,29]. Thus, further efforts should be made to establish the exact mechanism of miR-21 as a diagnostic and prognostic epigenetic tool in HCV infection.

Expression of miR-122 in our study was elevated in HCV patients, regardless of DAA therapy response, and correlated with cytolysis through AST and ALT levels in the acute phase of HCV infection. This marker is liver-specific and is a critical requisite for efficient HCV replication by interacting with 5`-non-coding regions of HCV to maintain RNA abundance and shape ribosomal binding sites [30]. As such, miR-122 levels are thought to correlate with the replicative potential of HCV within infected hepatocytes. Existing clinical research on miR-122 in HCV infection points to a larger issue of normalization in circulating microRNA assays, which precludes standardization and accounts for variable or even conflicting derivations. Numerous studies have correlated serum microRNA-122 and ALT levels, suggesting it can be used to identify liver injury secondary to chronic HCV infection [31–33]. However, the opposite was also reported in chronic HCV and hepatitis B virus infections [34,35].

In chronic HCV infection patients with persistent cytolysis syndrome, Bihrer *et al*. reported no differences in serum miR-122 levels compared to healthy controls, while van der Meer *et al*. found a 12-fold increase in this marker HCV patients [32,33]. Trebicka *et al*. found that as ALT rises in later fibrosis stages in chronic HCV infection, miR-122 levels decrease [36]. This contradicts the findings of Matsuura *et al*. who concluded that miR-122 levels do not correlate with histologic findings of fibrosis [37].

Additionally, our study observed that the levels of miR-122 were maintained high, even in those patients with SVR after DAA therapy, leading us to conclude that the virus could maintain its replicative potential, despite its low plasma concentration.

Circulating miR-885 is not as extensively characterized as miR-122. In our study, miR-885 was up-regulated in the acute phase of HCV infection and downregulated secondary to DAA therapy. Furthermore, miR-885 was correlated with AST levels in a replicative phase of

HCV infection. Our data follow Gui *et al*., who found that miR-885 levels were increased in patients with chronic hepatitis, cirrhosis, and HCC, suggesting this molecule may serve as a general marker for liver-associated pathologies with better diagnostic performance than ALT [38]. Another study identified elevations in miR-885 during acute HCV infection, consistent with our results [39]. Based on these findings, elevations in miR-885 likely represent a conserved response to hepatic cytolysis during acute replication stages. Interestingly, miR-885 could serve as an epigenetic marker for therapy response in HCV infection due to its low levels found in our patients after successful therapy.

4. Conclusions

Although further clinical studies are needed to confirm our results, we revealed an innovative panel of epigenetic biomarkers in HCV-infected patients and their variation after successful DAA therapy. Moreover, we emphasized these markers' diagnostic and predictive potential correlated with the standard biochemical and sonographic diagnostic strategies. Our study could increase clinician awareness of this topic by addressing this innovative epigenetic perspective as a candidate for further research in developing novel biomarkers for progression and response to DAA therapy in HCV-infected patients.

Funding

This research received no external funding.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Liberto, M.C.; Marascio, N. Chronic HCV Infection: Clinical Advances and Eradication Perspectives. *J. Clin. Med* **2022**, *11*, https://doi.org/10.3390/jcm11020359.
2. Grebely, J.; Page, K.; Sacks-Davis, R.; van der Loeff, M.S.; Rice, T.M.; Bruneau, J.; Morris, M.D.; Hajarizadeh, B.; Amin, J.; Cox, A.L.; Kim, A.Y.; McGovern, B.H.; Schinkel, J.; George, J.; Shoukry, N.H.; Lauer, G.M.; Maher, L.; Lloyd, A.R.; Hellard, M.; Dore, G.J.; Prins, M.; the In, C.S.G. The Effects of Female Sex, Viral Genotype, and IL28B Genotype on Spontaneous Clearance of Acute Hepatitis C Virus Infection. *Hepatology* **2014**, *59*, 109–120, https://doi.org/10.1002/hep.26639.
3. Bruno, S.; Crosignani, A.; Maisonneuve, P.; Rossi, S.; Silini, E.; Mondelli, M.U. Hepatitis C Virus Genotype 1b as a Major Risk Factor Associated with Hepatocellular Carcinoma in Patients with Cirrhosis: A Seventeen-Year Prospective Cohort Study. *Hepatology* **2007**, *46*, 1350–1356, https://doi.org/10.1002/hep.21826.
4. Ly, K.N.; Xing, J.; Klevens, R.M.; Jiles, R.B.; Ward, J.W.; Holmberg, S.D. The Increasing Burden of Mortality from Viral Hepatitis in the United States between 1999 and 2007. *Ann Intern Med* **2012**, *156*, 271–278, https://doi.org/10.7326/0003-4819-156-4-201202210-00004.
5. Isac, T.; Isac, S.; Ioanitescu, S.; Mihaly, E.; Tanasescu, M.-D.; Balan, D.G.; Tulin, A.; Iliescu, L. Dynamics of Serum α-Fetoprotein in Viral Hepatitis C without Hepatocellular Carcinoma. *Exp Ther Med* **2021**, *22*, https://doi.org/10.3892/etm.2021.10181.
6. Isac, S.; Panaitescu, A.M.; Iesanu, M.I.; Zeca, V.; Cucu, N.; Zagrean, L.; Peltecu, G.; Zagrean, A.-M. Maternal Citicoline-Supplemented Diet Improves the Response of the Immature Hippocampus to Perinatal Asphyxia in Rats. *Neonatology* **2020**, *117*, 729–735, https://doi.org/10.1159/000512145.
7. Wu, J.; Nagy, L.E.; Liangpunsakul, S.; Wang, L. Non-Coding RNA Crosstalk with Nuclear Receptors in Liver Disease. *Biochim Biophys Acta Mol Basis Dis* **2021**, *1867*, https://doi.org/10.1016/j.bbadis.2021.166083.

8. Gim, J.-A.; Bang, S.M.; Lee, Y.-S.; Lee, Y.; Yim, S.Y.; Jung, Y.K.; Kim, H.; Kim, B.-H.; Kim, J.H.; Seo, Y.S.; Yim, H.J.; Yeon, J.E.; Um, S.H.; Byun, K.S. Evaluation of the severity of nonalcoholic fatty liver disease through analysis of serum exosomal miRNA expression. *PLOS ONE* **2021**, *16*, https://doi.org/10.1371/journal.pone.0255822.
9. Mahmoudi, A.; Butler, A.E.; Jamialahmadi, T.; Sahebkar, A. The role of exosomal miRNA in nonalcoholic fatty liver disease. *Journal of Cellular Physiology* **2022**, *237*, 2078-2094, https://doi.org/10.1002/jcp.30699.
10. Bardhi, E.; McDaniels, J.; Rousselle, T.; Maluf, D.G.; Mas, V.R. Nucleic Acid Biomarkers to Assess Graft Injury after Liver Transplantation. *JHEP Rep* **2022**, *4*, https://doi.org/10.1016/j.jhepr.2022.100439.
11. Waldron, P.R.; Holodniy, M. MicroRNA and Hepatitis C Virus- Challenges in Investigation and Translation: A Review of the Literature. *Diagnostic Microbiology and Infectious Disease* **2014**, *80*, 1–12, https://doi.org/10.1016/j.diagmicrobio.2014.05.024.
12. López-Sánchez, G.N.; Dóminguez-Pérez, M.; Uribe, M.; Chávez-Tapia, N.C.; Nuño-Lámbarri, N. Non-Alcoholic Fatty Liver Disease and MicroRNAs Expression, How It Affects the Development and Progression of the Disease. *Ann Hepatol* **2021**, *21*, https://doi.org/10.1016/j.aohep.2020.04.012.
13. Sanceau, J.; Gougelet, A. Chapter 11 - Noncoding RNAs in Liver Cancer Patients. In: *Clinical Applications of Noncoding RNAs in Cancer*. Gupta, S.C.; Challagundla, K.B. Eds.; Academic Press, **2022**; pp. 343–389.
14. Gramantieri, L.; Giovannini, C.; Piscaglia, F.; Fornari, F. MicroRNAs as Modulators of Tumor Metabolism, Microenvironment, and Immune Response in Hepatocellular Carcinoma. *J Hepatocell Carcinoma* **2021**, *8*, 369–385, https://doi.org/10.2147/JHC.S268292.
15. Mehta, A.; Baltimore, D. MicroRNAs as Regulatory Elements in Immune System Logic. *Nat Rev Immunol* **2016**, *16*, 279–294, https://doi.org/10.1038/nri.2016.40.
16. Luna, J.M.; Scheel, T.K.H.; Danino, T.; Shaw, K.S.; Mele, A.; Fak, J.J.; Nishiuchi, E.; Takacs, C.N.; Catanese, M.T.; de Jong, Y.P.; Jacobson, I.M.; Rice, C.M.; Darnell, R.B. Hepatitis C Virus RNA Functionally Sequesters miR-122. *Cell* **2015**, *160*, 1099-1110, https://doi.org/10.1016/j.cell.2015.02.025.
17. Loureiro, D.; Tout, I.; Narguet, S.; Benazzouz, S.M.; Mansouri, A.; Asselah, T. MiRNAs as Potential Biomarkers for Viral Hepatitis B and C. *Viruses* **2020**, *12*, https://doi.org/10.3390/v12121440.
18. Xie, F.; Xiao, P.; Chen, D.; Xu, L.; Zhang, B. MiRDeepFinder: A MiRNA Analysis Tool for Deep Sequencing of Plant Small RNAs. *Plant Mol Biol* **2012**, https://doi.org/10.1007/s11103-012-9885-2.
19. EASL Recommendations on Treatment of Hepatitis C: Final Update of the Series(☆). *J Hepatol* **2020**, *73*, 1170–1218, https://doi.org/10.1016/j.jhep.2020.08.018.
20. Ghany, M.G.; Morgan, T.R.; AASLD-IDSA Hepatitis C Guidance Panel. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases–Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Hepatology* **2020**, *71*, 686–721, https://doi.org/10.1002/hep.31060.
21. Singaravelu, R.; Quan, C.; Powdrill, M.H.; Shaw, T.A.; Srinivasan, P.; Lyn, R.K.; Alonzi, R.C.; Jones, D.M.; Filip, R.; Russell, R.S.; Pezacki, J.P. MicroRNA-7 mediates cross-talk between metabolic signaling pathways in the liver. *Scientific Reports* **2018**, *8*, 361–361, https://doi.org/10.1038/s41598-017-18529-x.
22. Popoviciu, M.S.; Marin, V.N.; Vesa, C.M.; Stefan, S.D.; Stoica, R.A.; Serafinceanu, C.; Merlo, E.M.; Rizvi, A.A.; Rizzo, M.; Busnatu, S.; Stoian, A.P. Correlations between Diabetes Mellitus Self-Care Activities and Glycaemic Control in the Adult Population: A Cross-Sectional Study. *Healthcare* **2022**, *10*, https://doi.org/10.3390/healthcare10010174.
23. Vespasiani-Gentilucci, U.; Gallo, P.; De Vincentis, A.; Galati, G.; Picardi, A. Hepatitis C Virus and Metabolic Disorder Interactions towards Liver Damage and Atherosclerosis. *World J Gastroenterol* **2014**, *20*, 2825–2838, https://doi.org/10.3748/wjg.v20.i11.2825.
24. Han, S.; Zhang, T.; Kusumanchi, P.; Huda, N.; Jiang, Y.; Liangpunsakul, S.; Yang, Z. Role of MicroRNA-7 in Liver Diseases: A Comprehensive Review of the Mechanisms and Therapeutic Applications. *J Investig Med* **2020**, *68*, https://doi.org/10.1136/jim-2020-001420.
25. Marquez, R.T.; Bandyopadhyay, S.; Wendlandt, E.B.; Keck, K.; Hoffer, B.A.; Icardi, M.S.; Christensen, R.N.; Schmidt, W.N.; McCaffrey, A.P. Correlation between MicroRNA Expression Levels and Clinical Parameters Associated with Chronic Hepatitis C Viral Infection in Humans. *Lab Invest* **2010**, *90*, 1727–1736, https://doi.org/10.1038/labinvest.2010.126.
26. Bihrer, V.; Waidmann, O.; Friedrich-Rust, M.; Forestier, N.; Susser, S.; Haupenthal, J.; Welker, M.; Shi, Y.; Peveling-Oberhag, J.; Polta, A.; von Wagner, M.; Radeke, H.H.; Sarrazin, C.; Trojan, J.; Zeuzem, S.; Kronenberger, B.; Piiper, A. Serum MicroRNA-21 as Marker for Necroinflammation in Hepatitis C Patients with and without Hepatocellular Carcinoma. *PLOS ONE* **2011**, *6*, https://doi.org/10.1371/journal.pone.0026971.
27. Marquez, R.T.; Wendlandt, E.; Galle, C.S.; Keck, K.; McCaffrey, A.P. MicroRNA-21 Is Up-regulated during the Proliferative Phase of Liver Regeneration, Targets Pellino-1, and Inhibits NF-KappaB Signaling. *Am J Physiol Gastrointest Liver Physiol* **2010**, *298*, G535-541, https://doi.org/10.1152/ajpgi.00338.2009.
28. Xu, J.; Wu, C.; Che, X.; Wang, L.; Yu, D.; Zhang, T.; Huang, L.; Li, H.; Tan, W.; Wang, C.; Lin, D. Circulating MicroRNAs, MiR-21, MiR-122, and MiR-223, in Patients with Hepatocellular Carcinoma or Chronic Hepatitis. *Mol Carcinog* **2011**, *50*, 136–142, https://doi.org/10.1002/mc.20712.

29. Zhang, Y.; Jia, Y.; Zheng, R.; Guo, Y.; Wang, Y.; Guo, H.; Fei, M.; Sun, S. Plasma MicroRNA-122 as a Biomarker for Viral-, Alcohol-, and Chemical-Related Hepatic Diseases. *Clinical Chemistry* **2010**, *56*, 1830–1838, https://doi.org/10.1373/clinchem.2010.147850.
30. Schult, P.; Roth, H.; Adams, R.L.; Mas, C.; Imbert, L.; Orlik, C.; Ruggieri, A.; Pyle, A.M.; Lohmann, V. MicroRNA-122 Amplifies Hepatitis C Virus Translation by Shaping the Structure of the Internal Ribosomal Entry Site. *Nat Commun* **2018**, *9*, 2613, https://doi.org/10.1038/s41467-018-05053-3.
31. Waidmann, O.; Bihrer, V.; Pleli, T.; Farnik, H.; Berger, A.; Zeuzem, S.; Kronenberger, B.; Piiper, A. Serum MicroRNA-122 Levels in Different Groups of Patients with Chronic Hepatitis B Virus Infection. *J Viral Hepat* **2012**, *19*, e58-65, https://doi.org/10.1111/j.1365-2893.2011.01536.x.
32. Bihrer, V.; Friedrich-Rust, M.; Kronenberger, B.; Forestier, N.; Haupenthal, J.; Shi, Y.; Peveling-Oberhag, J.; Radeke, H.H.; Sarrazin, C.; Herrmann, E.; Zeuzem, S.; Waidmann, O.; Piiper, A. Serum MiR-122 as a Biomarker of Necroinflammation in Patients with Chronic Hepatitis C Virus Infection. *Am J Gastroenterol* **2011**, *106*, 1663–1669, https://doi.org/10.1038/ajg.2011.161.
33. van der Meer, A.J.; Farid, W.R.R.; Sonneveld, M.J.; de Ruiter, P.E.; Boonstra, A.; van Vuuren, A.J.; Verheij, J.; Hansen, B.E.; de Knegt, R.J.; van der Laan, L.J.W.; Janssen, H.L.A. Sensitive Detection of Hepatocellular Injury in Chronic Hepatitis C Patients with Circulating Hepatocyte-Derived MicroRNA-122. *J Viral Hepat* **2013**, *20*, 158–166, https://doi.org/10.1111/jvh.12001.
34. Wang, J.; Jiang, D.; Rao, H.; Zhao, J.; Wang, Y.; Wei, L. Absolute Quantification of Serum MicroRNA-122 and Its Correlation with Liver Inflammation Grade and Serum Alanine Aminotransferase in Chronic Hepatitis C Patients. *Int J Infect Dis* **2015**, *30*, 52–56, https://doi.org/10.1016/j.ijid.2014.09.020.
35. Ji, F.; Yang, B.; Peng, X.; Ding, H.; You, H.; Tien, P. Circulating MicroRNAs in Hepatitis B Virus-Infected Patients. *J Viral Hepat* **2011**, *18*, e242-251, https://doi.org/10.1111/j.1365-2893.2011.01443.x.
36. Trebicka, J.; Anadol, E.; Elfimova, N.; Strack, I.; Roggendorf, M.; Viazov, S.; Wedemeyer, I.; Drebber, U.; Rockstroh, J.; Sauerbruch, T.; Dienes, H.-P.; Odenthal, M. Hepatic and Serum Levels of MiR-122 after Chronic HCV-Induced Fibrosis. *J Hepatol* **2013**, *58*, 234–239, https://doi.org/10.1016/j.jhep.2012.10.015.
37. Matsuura, K.; Aizawa, N.; Enomoto, H.; Nishiguchi, S.; Toyoda, H.; Kumada, T.; Iio, E.; Ito, K.; Ogawa, S.; Isogawa, M.; Alter, H.J.; Tanaka, Y. Circulating Let-7 Levels in Serum Correlate With the Severity of Hepatic Fibrosis in Chronic Hepatitis C. *Open Forum Infect Dis* **2018**, *5*, https://doi.org/10.1093/ofid/ofy268.
38. Gui, J.; Tian, Y.; Wen, X.; Zhang, W.; Zhang, P.; Gao, J.; Run, W.; Tian, L.; Jia, X.; Gao, Y. Serum MicroRNA Characterization Identifies MiR-885-5p as a Potential Marker for Detecting Liver Pathologies. *Clin Sci (Lond)* **2011**, *120*, 183–193, https://doi.org/10.1042/CS20100297.
39. El-Diwany, R.; Wasilewski, L.N.; Witwer, K.W.; Bailey, J.R.; Page, K.; Ray, S.C.; Cox, A.L.; Thomas, D.L.; Balagopal, A. Acute Hepatitis C Virus Infection Induces Consistent Changes in Circulating MicroRNAs That Are Associated with Nonlytic Hepatocyte Release. *J Virol* **2015**, *89*, 9454–9464, https://doi.org/10.1128/JVI.00955-15.