Serum miR-181b-5p predicts ascites onset in patients with compensated cirrhosis

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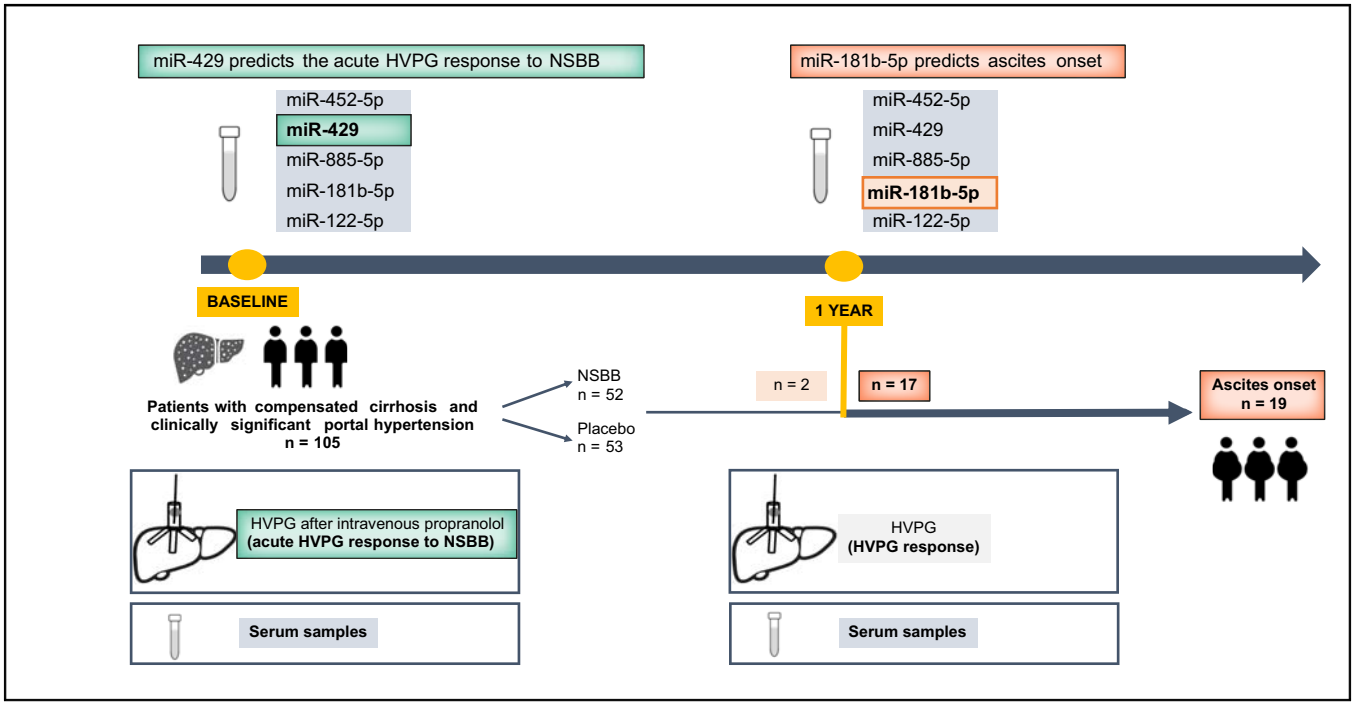
Graphical abstract



Highlights

- miR-181b-5p appears to be a useful serum biomarker to anticipate ascites onset.
- Low serum miR-181b-5p indicates low risk of ascites in compensated cirrhosis.
- Low serum miR-429 reflects acute hemodynamic response to non-selective beta-blockers.

Lay summary

Ascites marks the transition from the compensated to decompensated stage in cirrhosis and indicates a worsening in prognosis. There are currently no easily accessible tools to identify patients with compensated cirrhosis at risk of developing ascites. We evaluated the levels of novel molecules termed microRNAs in the blood of patients with compensated cirrhosis and observed that miR-181b-5p can predict which patients are going to develop ascites.

https://doi.org/10.1016/j.jhepr.2021.100368

Short communication

JHEP|Reports

Serum miR-181b-5p predicts ascites onset in patients with compensated cirrhosis

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JHEP Reports **2021.** https://doi.org/10.1016/j.jhepr.2021.100368

Background & Aims: Treatment with non-selective beta-blockers (NSBBs) reduces the risk of ascites, which is the most common decompensating event in cirrhosis. This study aimed to assess the ability of a serum microRNA (miRNA) signature to predict ascites formation and the hemodynamic response to NSBBs in compensated cirrhosis.
Methods: Serum levels of miR-452-5p, miR-429, miR-885-5p, miR-181b-5p, and miR-122-5p were analyzed in patients with compensated cirrhosis (N = 105). Hepatic venous pressure gradient (HVPG) was measured at baseline, after intravenous propranolol, and 1 year after randomization to NSBBs (n = 52) or placebo (n = 53) (PREDESCI trial). miRNAs were analyzed at baseline and at 1 year.
Results: Nineteen patients (18%) developed ascites, of whom 17 developed ascites after 1 year. miR-181b-5p levels at 1 year, but not at baseline, were higher in patients that developed ascites. The AUC of miR-181b-5p at 1 year to predict ascites was 0.7 (95% CI 0.59–0.78). miR-429 levels were lower at baseline in acute HVPG responders to NSBBs (AUC 0.65; 95% CI, 0.53–0.76), but levels at baseline and at 1 year were not associated with the HVPG response to NSBBs at 1 year.
Conclusions: Serum miR-181b-5p is a promising non-invasive biomarker to identify patients with compensated cirrhosis at risk of ascites development.
Lay summary: Ascites marks the transition from the compensated to decompensated stage in cirrhosis and indicates a worsening in prognosis. There are currently no easily accessible tools to identify patients with compensated cirrhosis at risk of developing ascites. We evaluated the levels of novel molecules termed microRNAs in the blood of patients with compensated cirrhosis and observed that miR-181b-5p can predict which patients are going to develop ascites.

Introduction

Ascites develops in 5%–10% of patients with compensated cirrhosis per year and represents the most frequent cause of decompensation.[1,2] Ascites results from an increase in portal pressure – estimated by the hepatic venous pressure gradient (HVPG) which must increase above 10 mmHg, *i.e.* clinically significant portal hypertension (CSPH) – and the development of circulatory dysfunction and renal sodium retention. The development of CSPH is a hallmark in the natural history of cirrhosis, since it places patients at risk of ascites and other complications of portal hypertension.[3] We currently lack other predictors of ascites formation and, in this regard, there is a clinical need for non-invasive biomarkers. A recent study has shown that non-

Keywords: Ascites; beta-blockers; cirrhosis; microRNA; portal hypertension.
Received 5 July 2021; received in revised form 19 August 2021; accepted 8 September 2021; available online 27 September 2021
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selective beta-blockers (NSBBs) reduce the risk of decompensation and death in patients with compensated cirrhosis and CSPH by approximately half.[2] The benefit of NSBBs was predominant in patients with HVPG decreasing >10% from baseline at 1 year.

MicroRNAs (miRNAs) are non-coding RNAs that regulate cellular processes by repressing the translation of mRNA.[4] miRNAs are secreted by cells and retrieved in body fluids which, in conjunction with their high stability, makes them attractive and accessible biomarkers.[5] Our group has recently identified a serum signature of 5 miRNAs – miR-452-5p, miR-429, miR-885-5p, miR-181b-5p, and miR-122-5p – in decompensated cirrhosis that differentiates patients with diuretic-sensitive from those with refractory ascites (through miR-181b-5p) and identifies those achieving an HVPG response to NSBBs (by means of miR-452-5p and miR-429).[6] A recent study has also characterized a set of circulating miRNAs that are altered in patients who progress to acute-on-chronic liver failure.[7] The aim of the present study was to evaluate our recently described serum miRNA signature as a biomarker to predict ascites formation and the HVPG response to NSBBs in a large cohort of patients with compensated cirrhosis and CSPH.

Patients and methods

Patients and study design

Serum samples were obtained from a registered collection (C.0005353, Collections Section, Biobank Registry of Instituto de Salud Carlos III, Madrid, Spain) of patients with compensated cirrhosis included in a randomized trial to evaluate the ability of NSBBs to prevent decompensation (PREDESCI trial; NCT01059396).[2] Cirrhosis was diagnosed on histological or compatible clinical, biochemical, and ultrasonographic findings. All patients' HVPG was measured at baseline and 1 year after being randomized to NSBBs or placebo. Only patients with baseline HVPG ≥10 mmHg were included. Patients were followed up to clinical decompensation (ascites, gastrointestinal bleeding, or overt hepatic encephalopathy) or death.

The novel miRNA signature – miR-452-5p, miR-429, miR-885-5p, miR-181b-5p, and miR-122-5p – was analyzed in serum samples collected and archived at baseline and at 1 year after randomization.[8] The study protocol adhered to the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Committee for Clinical Research (IRB number: 362/19). All participants granted written informed consent authorizing the storage and research use of their samples.

miRNA signature determination in serum samples

Extraction of total RNA and subsequent analysis of individual miRNAs by quantitative real-time PCR was performed following previously reported technical steps, and miR-103a-3p was also used as reference gene for data normalization.[6] The relative levels of each miRNA were calculated with the comparative Ct (cycle threshold) method: ΔCt, with ΔCt = Average $Ct_{tested\ miRNA}$ – Average $Ct_{miR\text{-}103a\text{-}3p}$ (average Ct = mean of the technical triplicate). ΔCt is inversely correlated with the amount of miRNA in the serum (*i.e.*, the higher the quantity or number of copies of miRNA, the lower the ΔCt). Detailed information is displayed in the supplementary materials and methods.

Statistical analysis

Sample size was not prespecified. Instead, we included all patients from the PREDESCI trial with archived serum samples collected at baseline and 1 year.[2] A multivariate Cox proportional hazards regression was used with adjustment for treatment arm and first-order interaction tested with a global likelihood ratio test. We plotted receiver-operating characteristic (ROC) curves and calculated the AUC and corresponding 95% CI to evaluate the diagnostic performances of the significant miRNAs. We performed a sensitivity analysis calculating time-to-event ROC curves in a survival data framework.[9] All analyses were 2-tailed and significance was set at p <0.01 after adjustment for multiple comparisons (supplementary materials and methods).

Results

Patient characteristics

We included 105 out of the 201 patients enrolled in the PREDESCI trial. The clinical characteristics of the study cohort and that of the patients excluded for lacking paired archived samples were similar (Table 1). Fifty-two (49.5%) and 53 (50.5%) patients belonged to the NSBB and placebo groups, respectively. The median follow-up was 36.2 months (IQR 26.3–47.6).

Serum miRNA levels and ascites formation

Of the 105 patients, 19 (18%) developed ascites during follow-up, 6 (11.5%) and 13 (24.5%) in the NSBB and placebo group, respectively. The median time to ascites formation was 26.3

Table 1. Baseline characteristics of the study population and of patients from the PREDESCI trial with no archived samples.

	Study population N = 105	No archived samples n = 96
Age (years)	65 (55–73)	60 (52–68)
Male sex	63 (60%)	60 (62.5%)
Cirrhosis etiology		
Alcohol	16 (15.2%)	17 (17.7%)
HCV	67 (63.8%)	46 (48%)
Alcohol and HCV	10 (9.5%)	7 (7.3%)
Other	12 (11.5%)	26 (27%)
Child-Pugh A/B/C	92/13/0	69/27/0
MELD score	6 (4–8)	5 (4-7)
Baseline HVPG (mmHg)	15 (12-18)	13 (11–16)
Esophageal varices at baseline		
None	40 (38%)	49 (51%)
Small	65 (62%)	47 (49%)
Treatment arm		
Placebo	53 (50.5%)	48 (50%)
NSBB	52 (49.5%)	48 (50%)
Propranolol/carvedilol	33/19	34/14
Decompensation		
Before 12 months follow-up		
Ascites	2	4
Hepatic encephalopathy	0	0
Variceal bleeding	0	2
Spontaneous bacterial peritonitis	0	1
Hepatorenal syndrome	0	0
After 12 months follow-up		
Ascites	17	6
Hepatic encephalopathy	4	5
Variceal bleeding	4	1
Spontaneous bacterial peritonitis	4	1
Hepatorenal syndrome	0	2

HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease; NSBBs, non-selective beta-blockers. Qualitative variables are provided as absolute values and percentages. Quantitative variables are provided as median (IQR).

months (IQR 21.2–38.5). Of the 19 patients, 17 (89.5%) developed ascites after 1 year.

The serum levels of all 5 miRNAs were reduced at 1 year (Fig. S1). Baseline miRNA levels did not associate with ascites development (Fig. S2). miR-181b-5p serum levels at 1 year were significantly higher in patients who eventually developed ascites than in those who did not (p = 0.001), with no differences in other miRNAs levels (Fig. 1).

miR-181b-5p did not correlate with the HVPG at 1 year (ρ = −0.09; p = 0.34). In survival analysis adjusted by treatment, miR-181b-5p remained significantly associated with ascites formation (hazard ratio 0.73; 95% CI 0.53–0.99; p = 0.04). No interaction with NSBBs was found (p value for first-order interaction = 0.27). The AUC of miR-181b-5p in a binary data framework to identify patients at risk of ascites was 0.7 (95% CI 0.59–0.78). When we performed a time-to-event analysis, the AUCs of ascites onset at 3, 6, 12, and 24 months after 1-year analysis were 0.92, 0.75, 0.70, and 0.41, respectively.

Serum miRNAs levels and the hemodynamic response to NSBB

miRNA levels showed no correlation with the HVPG at baseline (Table S2). An acute hemodynamic response after propranolol was achieved in 72 of the 105 patients (68.5%). Serum miR-429 baseline levels were lower in responders than in non-responders (p = 0.01) with no differences for the other miRNAs (Fig. S3). The AUC of miR-429 to identify acute response to NSBBs was 0.65 (95% CI 0.53–0.76).

miRNA levels at 1 year were similar in patients in the NSBB and placebo groups (Table S3). HVPG was reduced ≥10% from baseline at 1 year in 53% and 28% of the patients in the NSBB and placebo group, respectively (p = 0.015). In patients on NSBB, miRNAs levels at baseline and 1 year were not associated with the HVPG response at 1 year (Table S4).

Discussion

Our study shows that circulating miR-181b-5p is altered in patients with compensated cirrhosis and that its elevated serum levels are associated with ascites development. These findings extend the results of our previous study to compensated cirrhosis. Thus, miR-181b-5p is emerging as a novel non-invasive biomarker of ascites formation and progression through the stages of cirrhosis.

The goal of therapy in patients with compensated cirrhosis is to prevent decompensation. Accordingly, predictors of disease progression, specifically ascites formation, are hugely important. Circulating miRNAs have been extensively investigated in chronic liver disease[10] and, to a lesser extent, in decompensated cirrhosis[7,11] to determine their association with cirrhosis etiology and prognosis. However, we are unaware of studies addressing the value of circulating miRNAs in the transition from compensated to decompensated cirrhosis.

The serum levels of the 5 miRNAs comprising the signature significantly changed after 1 year of follow-up, illustrating their dynamic levels as cirrhosis evolves. However, only miR-181b-5p was associated with progression from compensated to decompensated cirrhosis. Indeed, miR-181b-5p levels predicted ascites occurrence since they were higher in individuals that developed ascites in subsequent months. As reinforced by the time-to-event analysis at different points, lower serum miR-181b-5p levels were associated with a low risk of ascites formation in the short-term. Notably, the predictive ability of miR-181b-5p in serum declined in parallel with the time elapsed to ascites onset, which is the most likely explanation of the absence of an association between baseline levels and ascites occurrence. We hypothesize that the greater ability to predict ascites appearance of miR-181b-5p levels at 1 year compared to those at baseline was due to the closer temporal relationship to the event of the

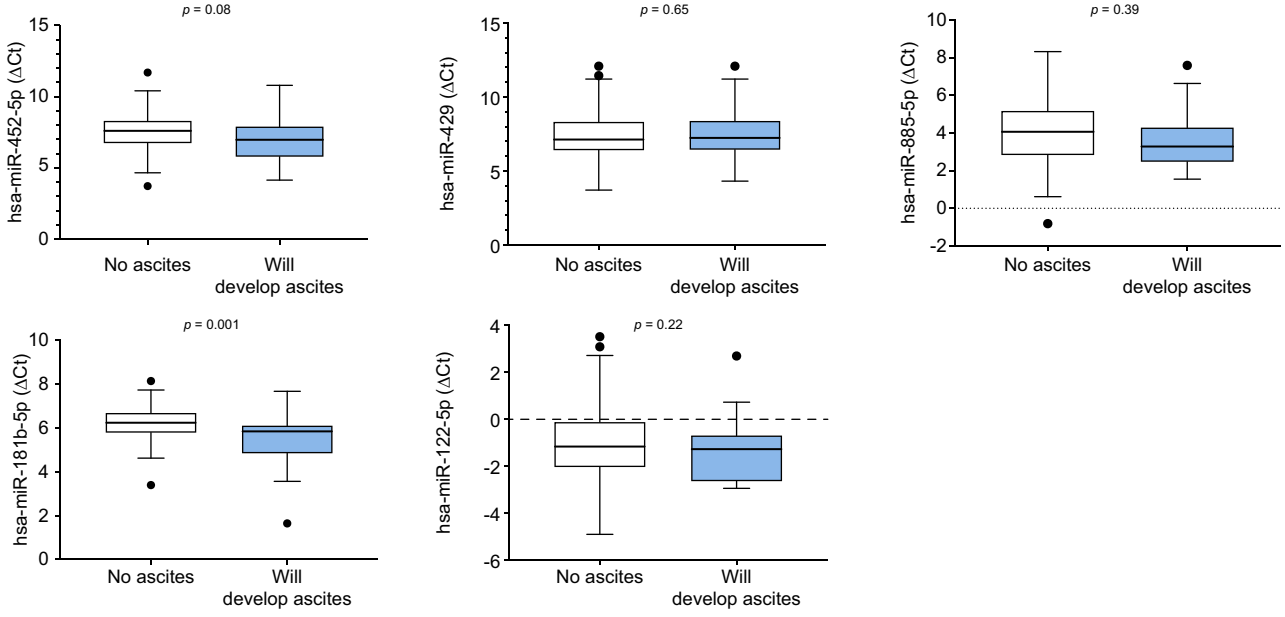

Fig. 1. MicroRNA serum levels at 1-year follow-up in patients who will and will not develop ascites. The middle horizontal line represents the median while the horizontal boundaries of the boxes represent the first and third quartiles. Levels of significance were assessed with the Mann-Whitney U test. ΔCt (cycle threshold) is inversely correlated with the amount of miRNA in the serum (*i.e.*, the higher the quantity or number of copies of miRNA in the sample, the lower the ΔCt).

former. Furthermore, NSBBs did not appear to influence the predictive ability of miR-181b-5p.

Previous evidence could provide some clues to understand the association between serum miR-181b-5p and ascites onset. Ascites in cirrhosis results from interrelated mechanisms, including progressive portal hypertension, endothelial dysfunction, and functional renal impairment. In this regard, miR-181b-5p seems involved in endothelial dysfunction and cardiovascular remodeling in different settings, such as vascular stiffness signaling in arterial hypertension,[12] diabetic cardiomyopathy development in mice,[13] and progression of abdominal aortic aneurysms.[14] In cirrhosis with ascites, we observed a direct correlation between circulating levels of miR-181b-5p and creatinine.[6] In the same line, miR-181b-5p, along with other circulating miRNAs, could be used to identify progression to acute-on-chronic liver failure in decompensated cirrhosis.[7] The value of circulating miR-181b-5p to predict cirrhosis progression is independent of etiology, since this property of miR-181b-5p has been identified in both alcohol- and virus-related cirrhosis.[6,7,15,16]

NSBBs reduce the risk of portal hypertension-related complications in compensated and decompensated cirrhosis.[2,17-19] The hemodynamic response to NSBBs is heterogeneous and we lack optimal non-invasive alternatives to assess it. In the present study, miR-429 levels were lower in patients who achieved an acute HVPG response to NSBBs, a finding in agreement with results in the decompensated setting, where NSBB responders at 1 month also showed lower levels of this miRNA.[6] Interestingly, miR-429 was not associated with the hemodynamic response to NSBBs in the long-term, a finding similar to the low correlation observed between baseline miR-181b-5p levels and ascites development. Taken together, these results suggest that the more distant the occurrence of the event of interest, the lower the predictive ability of the miRNA.

The results of this study represent a step forward in the search for innovative non-invasive biomarkers of cirrhosis progression and the response to NSBBs. In this study, we measured miRNA by the same robust and affordable technique described in decompensated cirrhosis.[6] We identified the same miRNA as predictors of disease progression (*i.e.*, miR-181b-5p) and response to NSBBs (*i.e.*, miR-429) in the compensated and decompensated settings, reinforcing the reproducibility of our results. We acknowledge some limitations. First, the analysis included only those patients from the original trial with archived samples at baseline and at 1 year suitable for analysis as well as with HVPG assessment at 1 year. However, a related bias seems unlikely since we did not find significant differences between the cohorts. Second, the number of patients that developed ascites was low. However, our study cohort was representative of the original trial in terms of treatment groups, ascites development, and hemodynamic response. Finally, our findings require external validation.

In summary, this study supports the usefulness of serum levels of miR-181b-5p as a non-invasive biomarker to anticipate ascites development in patients with compensated cirrhosis and CSPH who could benefit from a personalized follow-up.

Abbreviations

CSPH, clinically significant portal hypertension; Ct, cycle threshold; HVPG, hepatic venous pressure gradient; miRNAs, microRNAs; NSBBs, non-selective beta-blockers; ROC, receiver-operating characteristic.

Financial support

Supported by grants from the Ministerio de Ciencia e Innovación and Instituto de Salud Carlos III (SAF 2017-86343-R awarded to A.A., PI20/01302 to A.A., PI18/01901 to R.B., CIBEREHD-16PI03 and PI20/00220 to J.G.S.). A.G.G.P is the recipient of a grant from Ministerio de Ciencia e Innovación and Instituto de Salud Carlos III (Contrato Rio Hortega CM18/00091). Centro de Investigación Biomédica en Red en Enfermedades Hepáticas y Digestivas (CIBEREHD) is funded by the Instituto de Salud Carlos III with grants cofinanced by the European Development Regional Fund "A way to achieve Europe" (EDRF). Supported in part by a grant from Gilead Sciences (GLD19/00045).

Conflicts of interest

The authors have declared that no personal or financial competing interests exist.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

All authors have made substantial contributions and satisfy the criteria for authorship: conception and design; analysis and interpretation of the data; drafting of the article; critical revision of the article for important intellectual content; final approval of the article. All agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. We confirm that there are no other persons who satisfied the criteria for authorship but are not listed. We understand that the Corresponding Author is the sole contact for the Editorial process. Conceptualization: AGGP, LGB, JGS, AA. Data curation: AGGP, CV, CB, JG, NM, JCGP, JLC, CA, RM, MP, BP, SA, JGA, EA, FR, LGB, JMFP, RB, JB, JGS, AA. Formal analysis: AGGP, LGB, JGS, AA. Funding acquisition: AA, JGS, RB. Investigation: AGGP, LGB, JGS, AA. Methodology: AGGP, LGB, JGS, AA. Supervision: AA, JGS. Drafting of the manuscript: AGGP, LGB, JGS, AA. Critical revision of the manuscript for important intellectual content: AGGP, CV, CB, JG, NM, JCGP, JLC, CA, RM, MP, BP, SA, JGA, EA, FR, LGB, JMFP, RB, JB, JGS, AA.

Data availability statement

The data shown in this article are available from the corresponding authors upon request.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2021.100368.

References

Author names in bold designate shared co-first authorship

[1] D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. J Hepatol 2006;44(1):217–231. https://doi.org/10.1016/j.jhep.2005.10.013.

[2] **Villanueva C**, Albillos A, Genesca J, Garcia-Pagan JC, Calleja JL, Aracil C, et al. Beta blockers to prevent decompensation of cirrhosis in patients with clinically significant portal hypertension (PREDESCI): a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2019;393(10181):1597–1608. https://doi.org/10.1016/S0140-6736(18)31875-0.

[3] Ripoll C, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, Planas R, et al. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. Gastroenterology 2007;133(2):481–488. https://doi.org/10.1053/j.gastro.2007.05.024.

[4] Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH. An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. J Cell Physiol 2019;234(5):5451–5465. https://doi.org/10.1002/jcp.27486.

[5] Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;18(10):997–1006. https://doi.org/10.1038/cr.2008.282.
[6] **Garcia Garcia de Paredes A**, **Manicardi N**, **Tellez L**, Ibanez L, Royo F, Bermejo J, et al. Molecular profiling of decompensated cirrhosis by a novel MicroRNA signature. Hepatol Commun 2021;5(2):309–322. https://doi.org/10.1002/hep4.1642.
[7] Blaya DPE, Coll M, Lozano JJ, Graupera I, Schierwagen R, et al. Profiling circulating microRNAs in patients with cirrhosis and acute-on-chronic liver failure. JHEP Rep 2021;3(2).
[8] Hernandez-Gea V, Aracil C, Colomo A, Garupera I, Poca M, Torras X, et al. Development of ascites in compensated cirrhosis with severe portal hypertension treated with beta-blockers. Am J Gastroenterol 2012;107(3):418–427. https://doi.org/10.1038/ajg.2011.456.
[9] Heagerty PJ, Lumley T, Pepe MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. Biometrics 2000;56(2):337–344. https://doi.org/10.1111/j.0006-341x.2000.00337.x.
[10] Loosen SH, Schueller F, Trautwein C, Roy S, Roderburg C. Role of circulating microRNAs in liver diseases. World J Hepatol 2017;9(12):586–594. https://doi.org/10.4254/wjh.v9.i12.586.
[11] Cisilotto J, do Amaral AE, Rosolen D, Rode MP, Silva AH, Winter E, et al. MicroRNA profiles in serum samples from Acute-On-Chronic Liver Failure patients and miR-25-3p as a potential biomarker for survival prediction. Sci Rep 2020;10(1):100. https://doi.org/10.1038/s41598-019-56630-5.
[12] Hori D, Dunkerly-Eyring B, Nomura Y, Biswas D, Steppan J, Henao-Mejia J, et al. miR-181b regulates vascular stiffness age dependently in part by regulating TGF-beta signaling. PloS One 2017;12(3):e0174108. https://doi.org/10.1371/journal.pone.0174108.
[13] Copier CU, Leon L, Fernandez M, Contador D, Calligaris SD. Circulating miR-19b and miR-181b are potential biomarkers for diabetic cardiomyopathy. Sci Rep 2017;7(1):13514. https://doi.org/10.1038/s41598-017-13875-2.
[14] Di Gregoli K, Mohamad Anuar NN, Bianco R, White SJ, Newby AC, George SJ, et al. MicroRNA-181b controls atherosclerosis and aneurysms through regulation of TIMP-3 and elastin. Circ Res 2017;120(1):49–65. https://doi.org/10.1161/CIRCRESAHA.116.309321.
[15] Yu F, Zhou G, Li G, Chen B, Dong P, Zheng J. Serum miR-181b is correlated with hepatitis B virus replication and disease progression in chronic hepatitis B patients. Dig Dis Sci 2015;60(8):2346–2352. https://doi.org/10.1007/s10620-015-3649-1.
[16] **Chen YJ**, **Zhu JM**, **Wu H**, Fan J, Zhou J, Hu J, et al. Circulating microRNAs as a fingerprint for liver cirrhosis. PloS One 2013;8(6):e66577. https://doi.org/10.1371/journal.pone.0066577.
[17] D'Amico G, Garcia-Pagan JC, Luca A, Bosch J. Hepatic vein pressure gradient reduction and prevention of variceal bleeding in cirrhosis: a systematic review. Gastroenterology 2006;131(5):1611–1624. https://doi.org/10.1053/j.gastro.2006.09.013.
[18] Albillos A, Banares R, Gonzalez M, Ripoll C, Gonzalez R, Catalina MV, et al. Value of the hepatic venous pressure gradient to monitor drug therapy for portal hypertension: a meta-analysis. Am J Gastroenterol 2007;102(5):1116–1126. https://doi.org/10.1111/j.1572-0241.2007.01191.x.
[19] Turco L, Villanueva C, La Mura V, Garcia-Pagan JC, Reiberger T, Genesca J, et al. Lowering portal pressure improves outcomes of patients with cirrhosis, with or without ascites: a meta-analysis. Clin Gastroenterol Hepatol 2020;18(2):313–327 e6. https://doi.org/10.1016/j.cgh.2019.05.050.