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

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Supplementary materials and methods

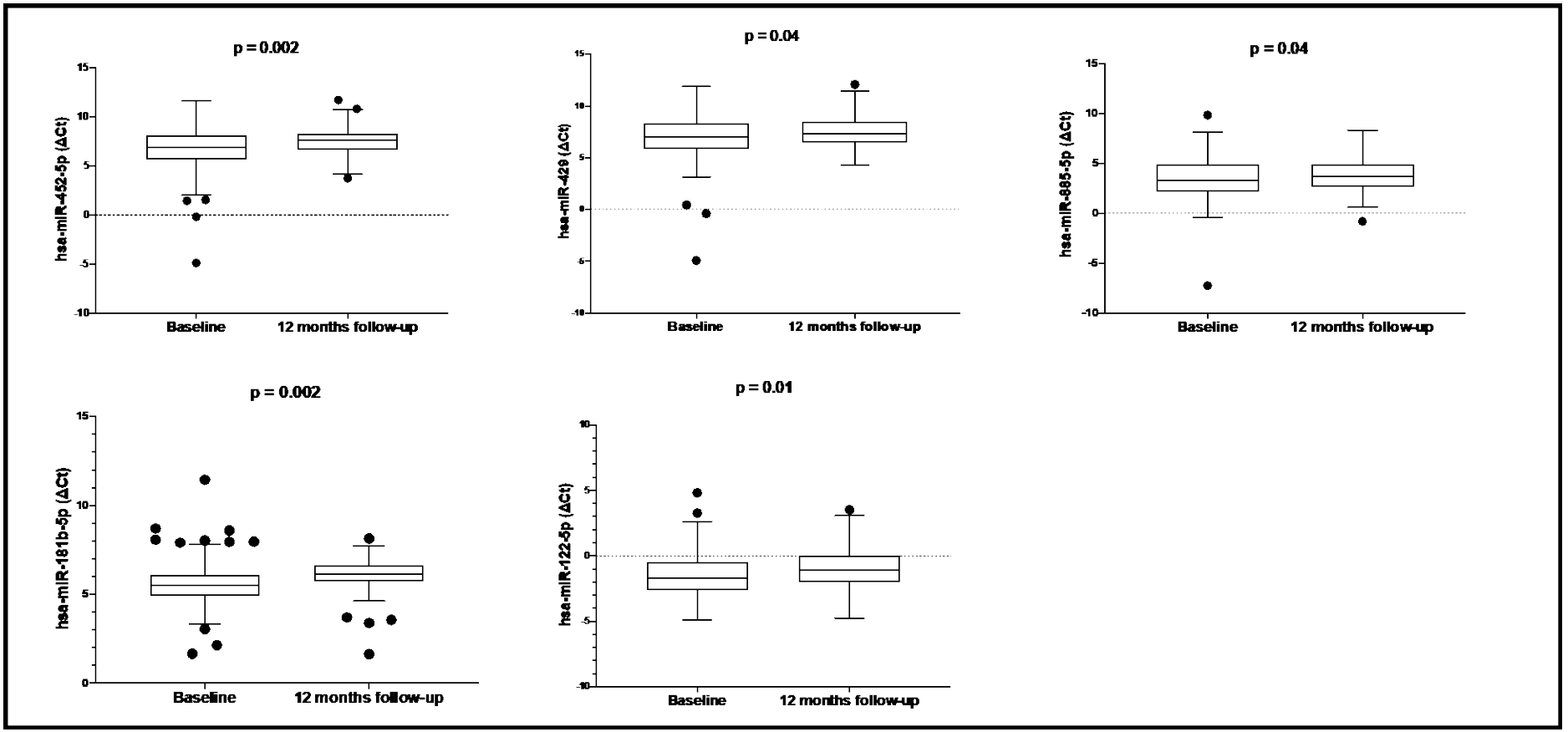
miRNA signature levels in serum samples

After collection, serum samples were centrifuged at 2500 × *g* for 15 min at room temperature, aliquoted, and stored at −80°C. For serum miRNA analysis, we first isolated total RNA from 200 µL of serum with the Plasma/Serum RNA Purification Mini Kit (#55000; Norgen Biotek, Thorold, Canada) according to the manufacturer´s protocol. Next, the levels of miRNAs were analyzed through amplification in individual quantitative real time-PCR reactions using commercially available locked nucleic acid probes specific for each miRNA of interest (miRNA primer sets from Exiqon A/S, Vedbæk, Denmark). All reactions were performed in triplicate with Light Cycler 480 equipment (Roche) following the supplier´s guidelines. The relative levels of each miRNA were calculated using miR-103a-3p as the housekeeping gene to minimize analytic variability and obtain a reliable and reproducible result. This reference gene was chosen for data normalization as it showed low standard deviation between all samples tested and it had been previously used for data normalization in the analysis of the miRNA signature in serum of patients with decompensated cirrhosis[1].

Statistical analysis

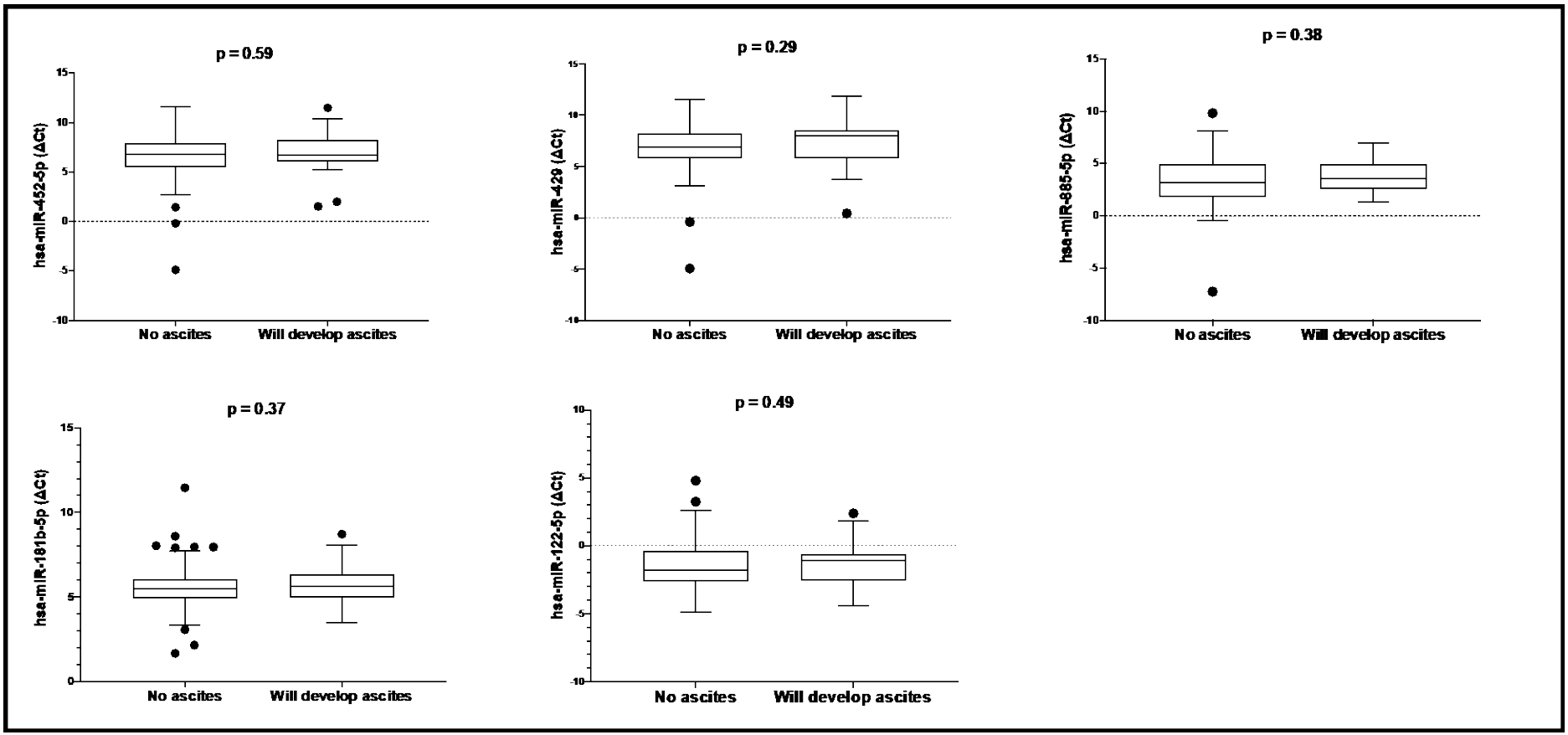
Categorical variables are displayed as absolute and relative frequencies. Quantitative variables are reported as mean and standard deviation or as median and interquartile range (IQR) when appropriate. To assess differences in miRNA serum levels, a two-sample t-test or non-parametric Mann–Whitney U test were used. Paired continuous data were assessed by a two-sample paired t-test. miRNA levels and HVPG were correlated by calculating the Spearman correlation coefficient (*rho*, ρ). We performed a complete case analysis given the low number of missing values (**Table S1**). Data were right-censored at the time of death, liver transplantation, last visit, or end of follow-up. The proportional hazards assumption was assessed via Schoenfeld residuals (test of proportional-hazards assumptions p = 0.97). Adjustment for multiple comparisons was performed by Bonferroni correction. Statistical analyses were performed using Stata/IC 14.2.

Fig S1. Change in microRNA serum levels: comparison between baseline and one-year assessment.



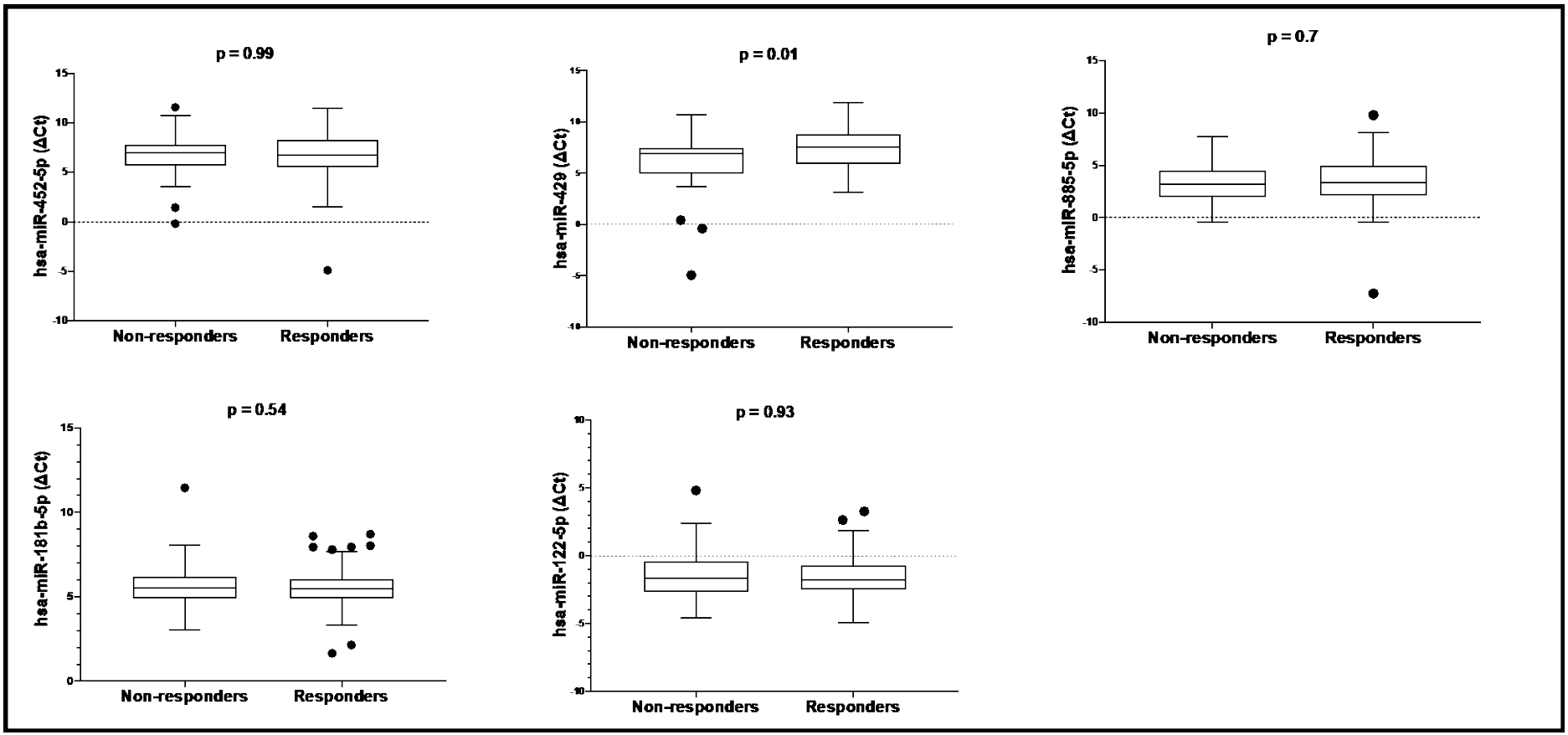
Middle horizontal line is the median and the horizontal boundaries of the boxes represent the first and third quartile. Levels of significance assessed with a two-sample paired t-test.

Fig S2. Baseline serum microRNA levels in patients that will and will not develop ascites during follow-up.



Middle horizontal line is the median and the horizontal boundaries of the boxes represent the first and third quartile. Levels of significance assessed with Mann-Whitney U-test.

Fig S3. MicroRNA serum levels at baseline in patients with and without acute hemodynamic response to intravenous propranolol.



Middle horizontal line represents the median and horizontal boundaries of the boxes the first and third quartile. Levels of significance assessed with a two-sample t-test (unequal variances).

Table S1. Missing values.

	N = 105
Baseline analysis	
miR-452-5p	1 (0.95%)
miR-429	2 (1.9%)
miR-885-5p	1 (0.95%)
miR-181b-5p	1 (0.95%)
miR-122-5p	1 (0.95%)
One-year analysis	
miR-452-5p	0 (0%)
miR-429	1 (0.95%)
miR-885-5p	0 (0%)
miR-181b-5p	0 (0%)
miR-122-5p	0 (0%)

Table S2. Baseline microRNA serum levels and baseline hepatic venous pressure gradient.

	HVPG at baseline (mmHg)
miR-452-5p	ρ= 0.08 p= 0.37
miR-429	ρ= 0.13 p= 0.19
miR-885-5p	ρ= -0.09 p= 0.34
miR-181b-5p	ρ= -0.04 p= 0.62
miR-122-5p	ρ= 0.06 p= 0.5

HVPG: hepatic venous pressure gradient. Spearman correlation coefficient (*rho*, ρ).

Table S3. Differences in miRNA serum levels at one year between patients in the non-selective beta-blocker group and the placebo group.

	p-value
miR-452-5p	0.65
miR-429	0.32
miR-885-5p	0.3
miR-181b-5p	0.56
miR-122-5p	0.18

Levels of significance assessed with a two-sample t-test (unequal variances).

Table S4. microRNAs assessed at baseline and at one-year follow-up and hemodynamic response at one-year in patients under non-selective beta-blocker treatment.

	p-value **Baseline levels & hemodynamic response at one-year**	**p-value** **One-year follow-up levels & hemodynamic response at one-year**
miR-452-5p	0.23	0.63
miR-429	0.68	0.17
miR-885-5p	0.73	0.85
miR-181b-5p	0.99	0.64
miR-122-5p	0.78	0.63

Levels of significance assessed with Mann-Whitney U-test.

Reference

1.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):309-22.