Metabolomic profiles to differentiate between porto-sinusoidal vascular disorder, cirrhosis, and health

Georg Semmler, Oleksandr Petrenko, Juanjo Jose Lozano, Sarah Shalaby, Juan I. Sánchez-Avila, Nara Marella, J. Thomas Hannich, Katharina Wöran, Lorenz Balcar, Benedikt Simbrunner, Katharina Lampichler, Behrang Mozayani, Michael Trauner, Mattias Mandorfer, Thomas Reiberger, Juan Carlos García Pagán, Bernhard Scheiner

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Supplementary methods. Metabolomics data acquisition and pre-processing.

Hydrophilic metabolites

An analytical method based on ion-pairing reversed-phase liquid chromatography and targeted dMRM was used. Absolute quantification of around 200 metabolites from central carbon metabolism, including glycolysis, pentose phosphate pathway, TCA cycle, amino acid, and nucleobase metabolism, as well as related metabolic pathways, was performed using heavy isotope-labeled as internal standards and external calibration curves. A 1290 Infinity II UHPLC system (Agilent Technologies) coupled with a 6470 triple quadrupole mass spectrometer (Agilent Technologies) was used for the LC-MS/MS analysis. The chromatographic separation for samples was carried out on a ZORBAX RRHD Extend-C18, 2.1 x 150 mm, 1.8 µm analytical column (Agilent Technologies). PeakBot software (vers. 0.9.54) was used for data processing[22].

Lipids

The LC-MS analysis was performed using a Vanquish UHPLC system (Thermo Fisher Scientific) combined with an Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer (Thermo Fisher Scientific). Lipid separation was performed by reversed-phase chromatography employing an Accucore C18, 2.6 µm, 150 x 2 mm (Thermo Fisher Scientific) analytical column at a column temperature of 35 °C. As mobile phase A an acetonitrile/water (50/50, v/v) solution containing 10 mM ammonium formate and 0.1 % formic acid was used. Mobile phase B consisted of acetonitrile/isopropanol/water (10/88/2, v/v/v) containing 10 mM ammonium formate and 0.1% formic acid. The flow rate was set to 400 µL/min. A gradient of mobile phase B was applied to ensure optimal separation of the analyzed lipid species. The mass spectrometer was operated in ESI-positive and -negative mode, capillary voltage 3500 V (positive) and 3000 V (negative), vaporize temperature 320 °C, ion transfer tube temperature 285 °C, sheath gas 60

arbitrary units, aux gas 20 arbitrary units and sweep gas 1 arbitrary unit. The Orbitrap MS scan mode at 120000 mass resolution was employed for lipid detection. The scan range was set to 250-1200 m/z for both positive and negative ionization modes, the AGC target was set to 2.0e5, and the intensity threshold to 5.0e3. The data analysis was performed using the TraceFinder software (ThermoFisher Scientific).

Fatty acids

Fatty acid analysis was based on the derivatization of free fatty acids, separation via reversed-phase liquid chromatography, and detection with a high mass accuracy mass spectrometer. Absolute quantification is based on external calibration and isotopically labeled internal standardization. Some minor fatty acids are quantified only in a semi-quantitative way. The LC-MS analysis was performed using a Vanquish UHPLC system (Thermo Fisher Scientific) combined with an Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer (Thermo Fisher Scientific). Fatty acid separation was performed by reversed-phase chromatography employing an Accucore C18, 2.6 µm, 150 x 2 mm (Thermo Fisher Scientific). The data analysis was performed using the TraceFinder software (ThermoFisher Scientific).

Fig. S1. Top five principal components (PC). No combination allows for perfect separation of PSVD from cirrhosis or samples from healthy volunteers, but PC1 and PC4 result in better distinguishing the patient metabolic profiles. **B:** Highest-ranked metabolites in PC1 and PC4**.**

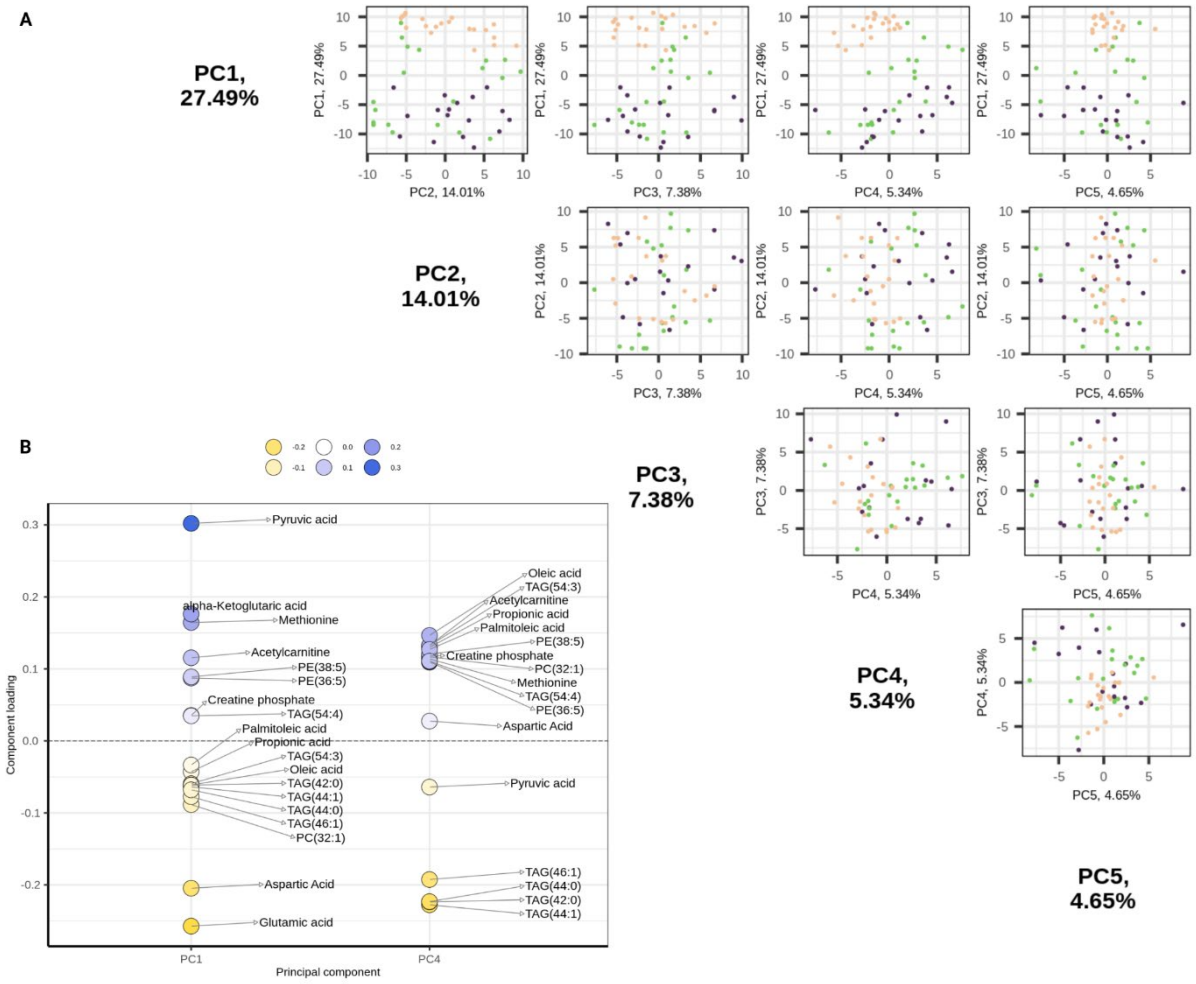

Fig. S2. A: Comparison of total serum triglycerides between the $PSVD_{subgroup}$ and other PSVD patients. B: Differential metabolite abundance testing results between $PSVD_{subgroup}$ and other PSVD patients.

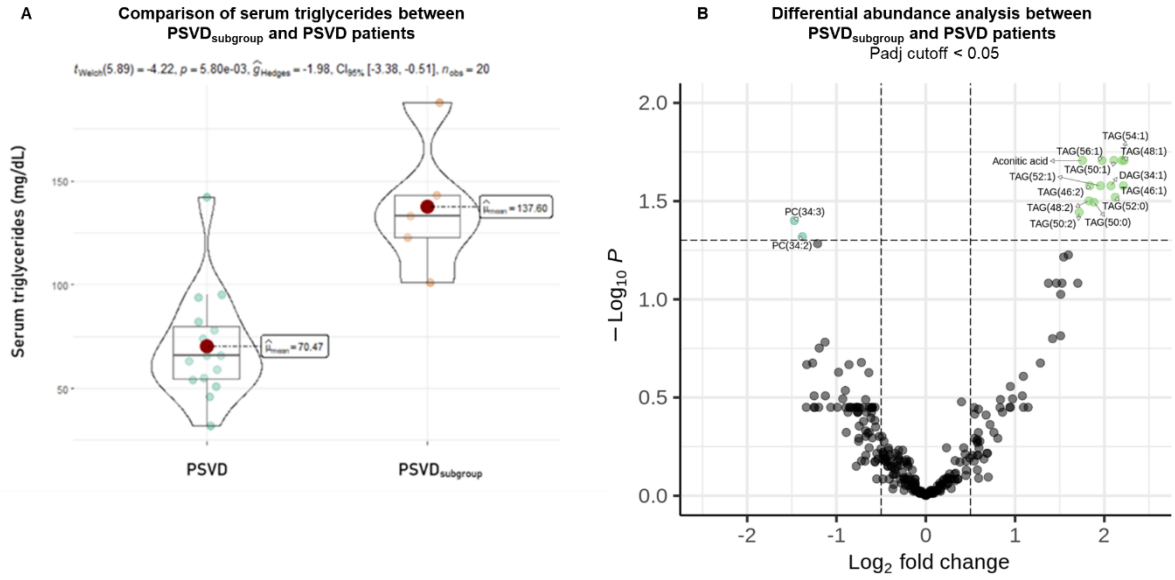

Fig. S3. Pattern analysis of missing data. All missing metabolites in more than 10% of samples (i.e., above the set data imputation threshold) and less than 100% of samples (i.e., present in at least one sample) are compared between the groups. A high number of missing values in one group, coded in yellow color, suggests a link between the respective group and concentration below the detection limit.

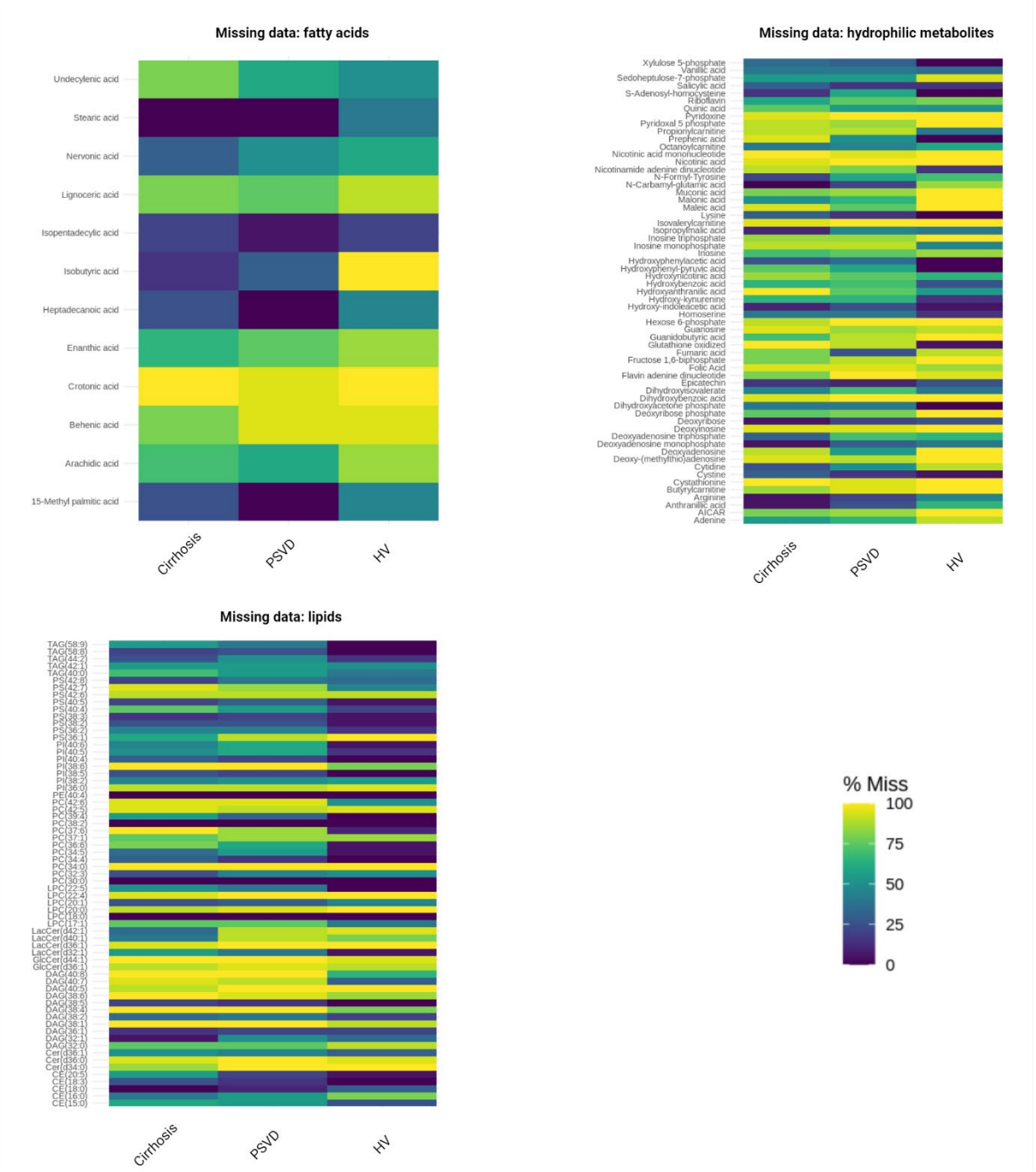

Fig. S4. Pyrimidine metabolic pathway in PSVD compared to the non-disease group. Color-coding indicates the direction of abundance: blue is linked to downregulation, while yellow indicates upregulation.

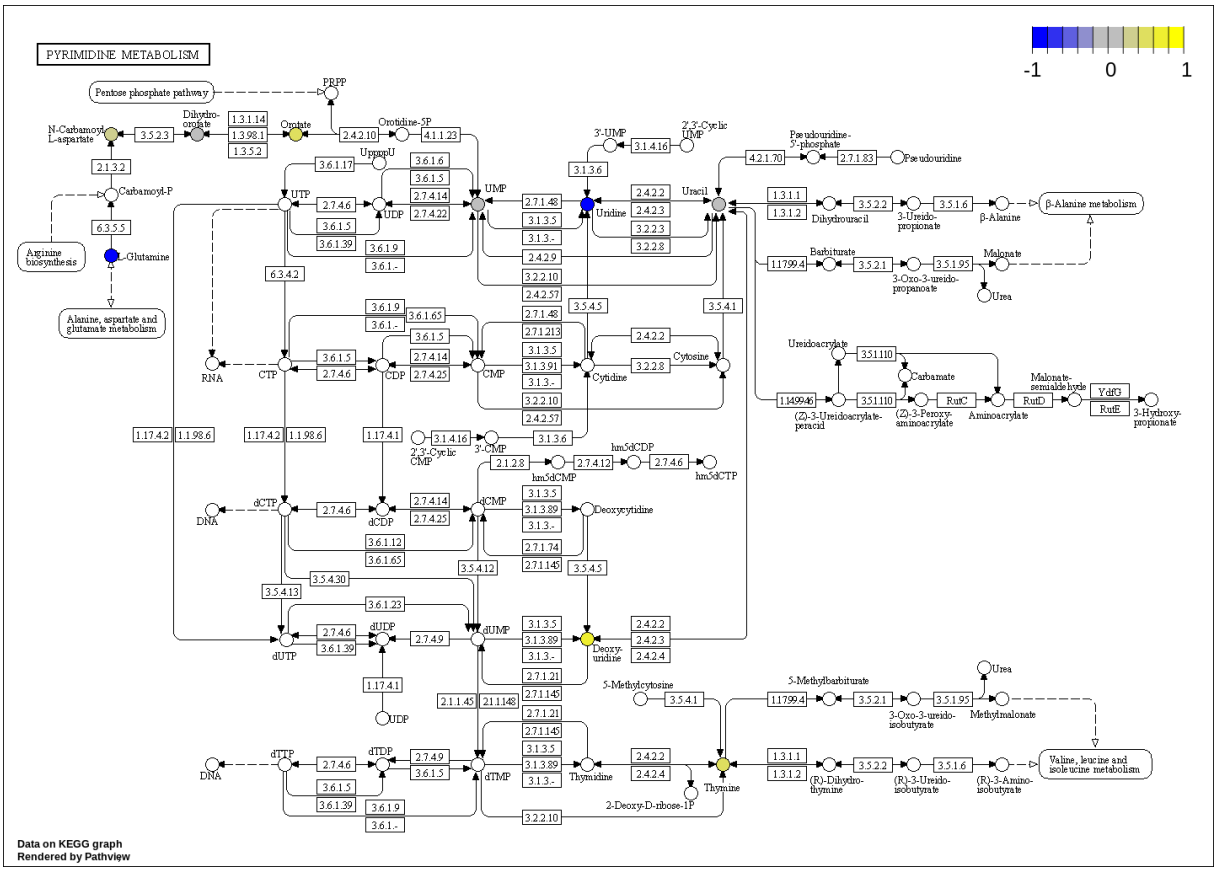

Fig. S5. Metabolites utilized for machine learning models**. A**: The flow chart demonstrates steps for metabolite prioritization for subsequent use in machine learning-based prediction. **B**: schematic representation of the artificial neural network used for classification in the study. The input layer (I_n) represents metabolites. Only one hidden (H_n) layer with five neurons is present to avoid overfitting. The outcome (O1) node represents the classification result. Bias nodes (B_n) represent constant values optimized for respective model fit.

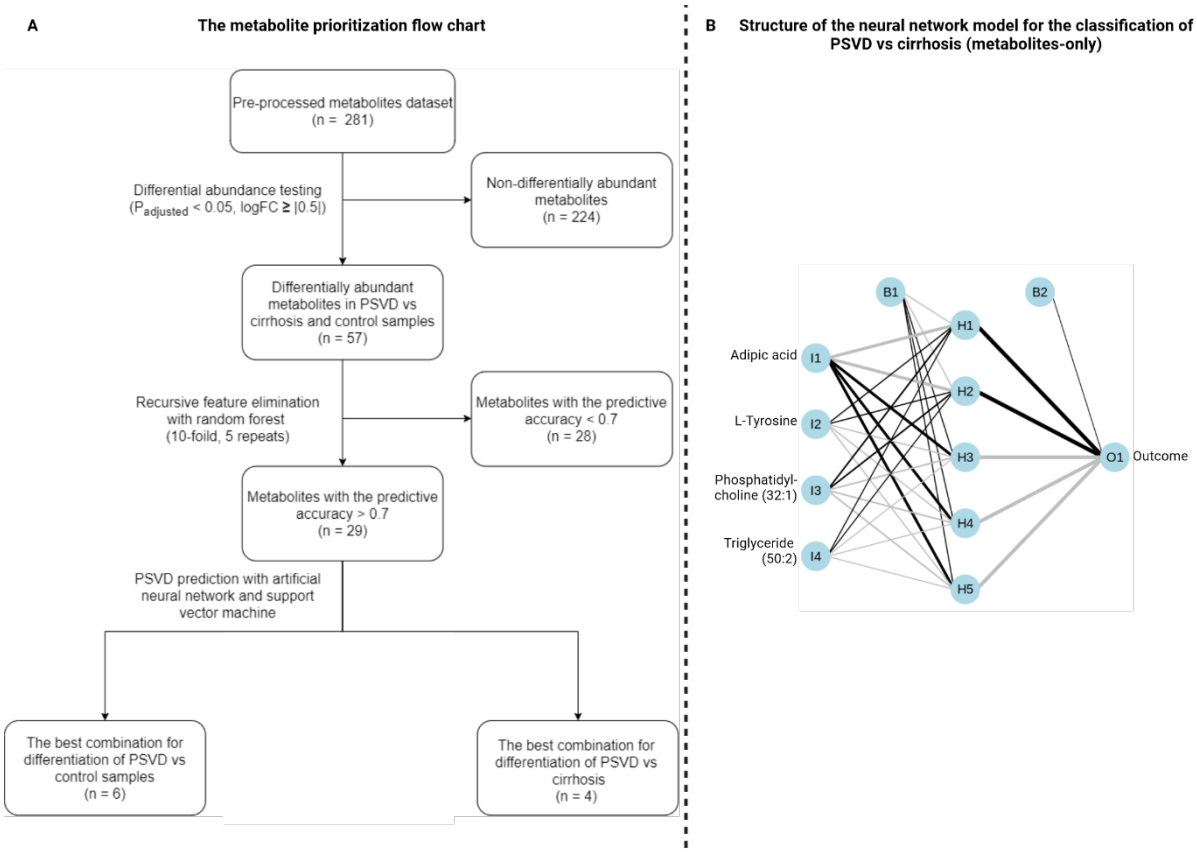

Fig. S6. Comparative analysis of the metabolites with the highest diagnostic performance in patients with PSVD and healthy volunteers in Vienna and Barcelona cohorts. Normalized and Z-scaled values were used for testing. Shapiro-Wilk test for normality was performed, following the Welch Two Sample t-test (normal distribution) or Wilcoxon rank sum test (non-normal distribution), results of which are reported on the plots. Ns: P > 0.05, *** : P ≤ 0.001.

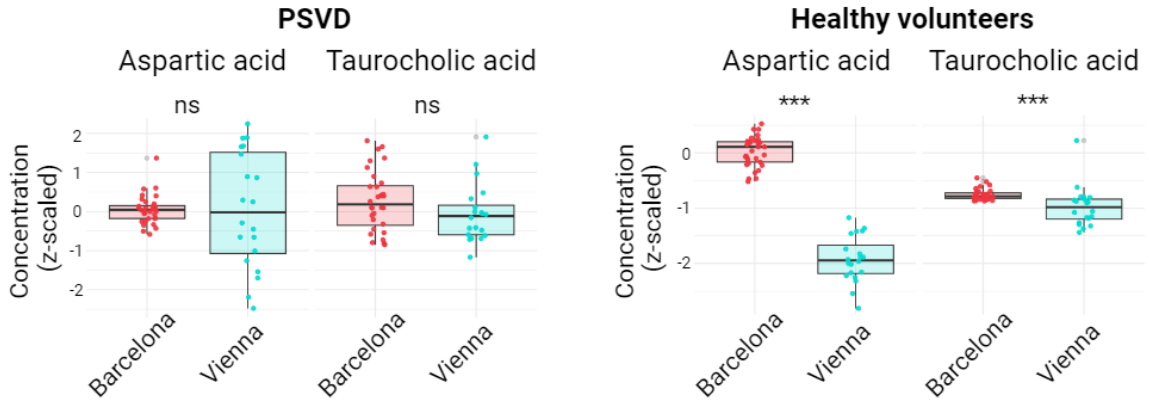

Table S1. Characteristics of patients with porto-sinusoidal vascular disorder (PSVD) and cirrhosis compared between the Vienna and the Barcelona cohort. Data are presented as a mean and standard deviation to align with the data from Seijo et al. (2015).

	Vienna cohort		**Barcelona cohort**	
	PSVD (n=20)	**Cirrhosis (n=20)**	**PSVD (n=34)**	**Cirrhosis (n=34)**
Age, years	44.8±13.5	53.1±10.0	47±19	61±10
Male sex	13 (65.0%)	16 (80.0%)	23 (67.6%)	23 (67.6%)
Ascites	2 (10.0%)	8 (40.0%)	11 (32.4%)	8 (23.5%)
Varices	19 (95.0%)	19 (95.0%)	32 (94.1%)	28 (82.4%)
Variceal bleeding	5 (25.0%)	4 (20.0%)	11 (32.4%)	6 (17.6%)
Hepatic encephalopathy	0 (0%)	3 (15.0%)	1 (2.9%)	0 (0%)
Child-Pugh Score, points	6.0±1.2	5.7±0.8	5.8±1.2	6.0±1.4
Child-Pugh stage A	14 (70.0%)	16 (80.0%)	26 (76.5%)	27 (79.4%)
Child-Pugh stage B/C	6 (30.0%)	4 (20.0%)	8 (23.5%)	7 (20.6%)
Platelet count, G/L	115±95	100±47	93±56	92±38.9
Prothrombin time ratio (%)	76±14	61±10	76±15	76±12
Albumin, g/dL	41.5±3.7	38.2±3.9	39±4.3	36±4.7
Bilirubin, mg/dL	1.0±0.8	0.8±0.2	1.0±1.3	1.4±0.8