CIRRHOSIS AND LIVER FAILURE

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Metabolomics discloses potential biomarkers to predict the acute HVPG response to propranolol in patients with cirrhosis

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Abstract

Background: In cirrhosis, a decrease in hepatic venous pressure gradient (HVPG) > 10% after acute iv propranolol (HVPG response) is associated with a lower risk of decompensation and death. Only a part of patients are HVPG responders and there are no accurate non-invasive markers to identify them. We aimed at discovering metabolomic biomarkers of HVPG responders to propranolol.

Methods: Sixty-six patients with cirrhosis and HVPG ≥ 10 mm Hg in whom the acute HVPG response to propranolol was assessed, were prospectively included. A targeted metabolomic serum analysis using ultrahigh-performance liquid chromatography coupled to mass spectrometry was performed. Different combinations of 2-3 metabolites identifying HVPG responders (HVPG reduction > 10%) were obtained by stepwise logistic regression. The best of these model (AUROC, Akaike criterion) underwent internal cross-validation and cut-offs to classify responders/non-responders was proposed.

Results: A total of 41/66 (62%) patients were HVPG responders. Three hundred and eighty-nine metabolites were detected and 177 were finally eligible. Eighteen metabolites were associated to the HVPG response at univariate analysis; at multivariable analysis, a model including a phosphatidylcholine (PC(P-16:0/22:6)) and a free fatty acid (20:2(n-6), eicosadienoic acid) performed well for HVPG response, with an AUROC of 0.801 (0.761 at internal validation). The cut-off 0.629 was the most efficient for overall classification (49/66 patients correctly classified). Two cut-off values allowed identifying responders (0.688, PPV 84%) and non-responders (0.384, NPV 82%) with undetermined values for 17/66 patients. Clinical variables did not add to the model.

Conclusions: The combination of two metabolites helps at identifying HVPG responders to acute propranolol. It could be a useful non-invasive test to classify the HVPG response to propranolol.

KEYWORDS
metabolite, non-selective beta-blockers, portal hypertension, variceal bleeding

1 | INTRODUCTION

Portal hypertension (PHT) is the driving force for decompensations occurring in cirrhosis. A portal pressure gradient ≥10 mm Hg as measured by the hepatic venous pressure gradient (HVPG) defines clinically significant PHT. Beyond this value the presence of oesophageal varices, ascites, hepatic encephalopathy and hepato-renal syndrome can appear; an HVPG ≥12 mm Hg is necessary for varices to bleed.[1] A decrease in HVPG ≥20% from baseline values or below 12 mm Hg spontaneously or under treatment with non-selective β-blockers (NSBB) is associated with less incidence of PHT-related complications and of death.[2-4]

The HVPG response to β-blocker therapy can also be determined with a single haemodynamic study,[5,6] where responders (about 50%-60%) are identified by decrease ≥10% from baseline after the acute administration of iv propranolol (0.15 mg/kg). As for the chronic response of HVPG, these patients have a lower incidence of decompensation and death.[5-7]

Several factors can influence the HVPG response to NSBB, including the degree of liver failure, the dose of β-blockers, the extent of portal-systemic collaterals and varices.[3,8-10] However, different parameters assessed to predict the HVPG response (heart rate, femoral/portal blood flow changes, β-adrenoceptors polymorphisms, antrum mucosa vasoactive proteins)[11-15] have not been accurate enough. Therefore, the invasive measurement of HVPG is necessary and, despite minimally invasive, some patients are reluctant to it and the technique is not universally available. Thus, it would be relevant to develop non-invasive methods for recognizing the response to β-blockers for medical therapy optimization.

Metabolomics is an "omics" discipline that has gained interest in biomedical research. The application of high-throughput techniques such as liquid chromatography coupled to mass spectrometry (LC-MS) allows measuring simultaneously thousands of metabolites from a variety of complex samples (biological fluids or tissue extracts) in a short-time period. These techniques perform a semi-quantitatively analysis of a wide range of molecules, such as glycerophospholipids, glycerolipids, sphingolipids, fatty acids, bile acids or amino acids. Recently, potential applications for metabolomics have been proposed by reporting specific profiles in several liver disorders such as drug-induced liver injury, NASH or idiopathic portal hypertension or to stratify degrees of cirrhosis severity.[16-22] Most of these early metabolomic analysis were untargeted and metabolites, defined based on their retention time and mass to charge ratio, could not always be identified. However, in the recent years, noteworthy advances in metabolomic field allow targeted analysis that identify specific metabolites, generating hypothesis and developing prognostic models to be used in clinical practice.

The aim of this pilot study was to identify a metabolomic serum profile in patients with cirrhosis and PHT that allows a non-invasive prediction of the HVPG response to acute iv propranolol.

Key Points

- Two serum metabolites help at identifying patients with cirrhosis and a good response to β-blockers for portal hypertension. It may help avoiding invasive studies to assess this response and may facilitate a better individualization of therapy.

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2 | PATIENTS AND METHODS

Sixty six patients with cirrhosis and clinically significant PHT (HVPG ≥ 10 mm Hg), in whom HVPG response to iv propranolol was assessed, were prospectively included between September 2010 and June 2015. All patients had oesophageal varices with or without a previous variceal bleeding and were to initiate primary or secondary prophylaxis with non-selective β-blockers. Inclusion criteria were diagnosis of cirrhosis (liver biopsy or unequivocal clinical data and compatible findings on imaging techniques); age between 18 and 80 years; HVPG ≥ 10 mm Hg; presence of oesophageal varices (with or without previous bleeding episode); and indication of β-blockers. Exclusion criteria were severe liver failure (Child-Pugh score>12 points); recent blood-derived product transfusion (patients with bleeding); hepatocellular carcinoma; acute alcoholic hepatitis; portal vein thrombosis; contraindications to β-blockers; pregnancy; or refusal to participate in this study. Hepatocellular carcinoma and acute alcoholic hepatitis were excluded because of their unknown effects on metabolome. All patients were on stable clinical conditions and patients with recent bleeding, this study was done at day 5 of admission and they were on clinical and haemodynamic stable conditions to initiate with β-blockers. This study was conducted following the principles of the Declaration of Helsinki (revised in Seoul in 2008). This study was approved by the Ethics Committee for Clinical Investigation of Hospital Clinic (registry number 2010/6008, approval 9/IX/10) and all patients gave their written informed consent.

Baseline clinical characteristics and laboratory tests were collected, as well the treatments received. Treatments were grouped in major families to control potential effects on metabolites profiles. The groups were: antibiotics, diuretics, antihypertensive drugs, insulin/oral antidiabetics and other hormones.

2.1 | Haemodynamic studies

The measurement of HVPG and its response to iv propranolol was performed as previously reported.[23] Briefly, after an overnight fast, under local anaesthesia (mepivacaine 1%, subcutaneously) with ultrasonographic guidance, an 8F venous catheter introducer (Axcess; Maxxim Medical, Athens, TX) was placed in the right jugular vein by the Seldinger technique. Under fluoroscopy, a 7F balloon-tipped catheter ("Fogarty" Edwards Lifesciences LLC, CA) was guided into the main right or middle hepatic vein for measurements of wedge (occluded,

WHVP) and free hepatic venous pressures (FHVP). HVPG results from the difference between WHVP and FHVP. The adequacy of occlusion was checked by gentle injection of a small amount of radiologic contrast medium after balloon inflation. After baseline measurements, iv propranolol (0.15 mg/kg) was administered over 10 minutes. HVPG response was assessed at minute 15-20 as previously described.[5,6] A positive HVPG response was defined as a decrease equal or greater than 10% from baseline value. All measurements were taken in triplicate and permanent tracings were obtained in each case in a multichannel recorder (GE Healthcare, Milwaukee, WI), and were reviewed specifically for this study by experienced investigators (JB, and JCGP).

2.2 | Blood sample details and metabolomic profiling

Blood samples were obtained prior to the haemodynamic studies. Peripheral blood was collected into a gel separator tube (Vacutainer system, Becton Dickinson, San Jose, CA; Ref 368969). The samples were centrifuged and aliquots of serum (250 μL) were frozen at −80°C until assayed at OWL Metabolomics.

Serum metabolic profiles were analysed as previously described. Briefly, UHPLC-single quadrupole-MS amino acid analysis system was combined with two separate UHPLC-time-of-flight (TOF)-MS-based platforms analysing methanol and chloroform/methanol serum extracts were combined. Identified ion features in the methanol extract platform included non-esterified fatty acids (FA), acyl carnitines, bile acids, steroids, oxidized FA, monoacylglycerophospholipids and monoetherglycerophospholipids. The chloroform/methanol extract platform provided coverage over glycerolipids, cholesteryl esters, sphingolipids, diacylglycerophospholipids, acyl-ether-glycerophospholipids and primary fatty acid amides. Lipid nomenclature follows the LIPID MAPS convention (www.lipidmaps.org). Full description of metabolite extraction methods and UHPLC-MS analysis of each platform is provided in Data S1.

All data were processed using the TargetLynx application manager for MassLynx 4.1 (Waters Corp.). The peak detection process included 389 metabolic features, identified prior to the analysis. Intrabatch normalization followed the procedure described by Martinez-Arranz et al (see Data S1).[24]

2.3 | Statistical analysis

2.3.1 | Metabolomic variables

We initially explored differences in metabolic profile of responders and non-responders after a Log2 fold-change transformation for each metabolite (Log2 FC = Log2(Average responders) – Log2(Average non-responders)). Log2 conversion makes data to become more normally distributed.[25] Thereafter, a univariate analysis by unpaired Student's t test (or Welch´s t test when unequal variances) was applied to assess differences among responders and non-responders. Heatmaps were created to show individual metabolite differences between groups.

After the metabolomic broad profiling analysis, only metabolites with a baseline chromatographic resolution and a good signal to noise ratio were considered for analysis and prognostic model development. This selection was done to build a robust model, easily reproducible and transferable to other laboratories worldwide. These metabolites underwent univariate logistic regression to assess HVPG response; those with a $P \leq 0.05$ underwent standard stepwise logistic regression to find predictive combinations of response. Based on the sample size, the proportion of responders and to avoid overfitting, we studied combinations of two or three metabolites. The performance of the models was assessed by means of AUROC curves and Akaike information criterion (AIC), which gives a relative value among potential models so that the best one has the lowest value.[26]

The best metabolite prognostic model was studied for potential cut-off points to identify HVPG responders and non-responders. Youden Index, sensitivity (SN), specificity (SP) and predictive values were assessed. The selected model underwent internal validation using "leave-one-out" cross-validation computing.[27]

The potential association of the significant metabolites and its potential influence by relevant clinical variables (aetiology, Child-Pugh class, prophylaxis type or medications) was further assessed.

2.3.2 | Clinical variables

Baseline variables were compared between HVPG responders and non-responders. Those clinical variables associated with the HVPG response were introduced in the metabolomic models attempting to improve their performance. Quantitative variables are expressed as mean ± standard deviation, and qualitative variables as absolute and relative frequencies. Categorical variables were compared using the chi-square test. Continuous variables were compared with Student's t test. Logistic regression was used for multivariable analysis.

Statistical analysis of clinical, laboratory and haemodynamic data was performed with the statistical package SPSS 20.0 (SPSS Inc, Chicago). Metabolic statistical analysis was performed with R statistical package v.3.1.0. Statistical significance was established at a $P < 0.05$.

3 | RESULTS

3.1 | Patients and haemodynamics

The clinical and laboratory characteristics of the 66 patients included in this study are summarized in Table 1. No baseline clinical, biochemical or haemodynamic variables were significantly different between HVPG responders and non-responders, except for a greater proportion of non-responders among patients to begin secondary prophylaxis. Forty-one (62%) patients were responders and 25 (38%) were non-responders. The mean HVPG was 16.9 ± 3.6 mm Hg with a mean decrease in responders of 21 ± 12%. Table 2 summarizes hepatic haemodynamic characteristics of patients.

TABLE 1 Baseline clinical characteristics of patients in this study

	Overall (N = 66)	Non-responders (n = 25)	Responders (n = 41)
Age (y)	60 ± 10	60 ± 10	59 ± 11
Male sex, n (%)	26 (65)	11 (73)	15 (60)
Aetiology alcohol/Viral/Others, n (%)	29(44)/27(41)/10(15)	13(52)/9(36)/3(12)	16(39)/18(44)/7(17)
1ry/2ry prophylaxis, n (%)	47 (71)/19(29)	14(56)/11(44)	33(81)/8(19)[a]
Child-Pugh Class A/B/C, n (%)	39(59)/16(24)/11(17)	13(52)/7(28)/5(20)	26(63)/9(22)/6(15)
MELD score, median (IQR)	10.9 (6)	10 (6.4)	11 (5.6)
Body mass index (kg/m^2)	26.8 ± 4.1	26.9 ± 4.4	26.8 ± 3.9
Glucose (mg/dL)	124 ± 45	133 ± 37	119 ± 49
Cholesterol (mg/dL)	144 ± 47	138 ± 36	148 ± 53
Triglycerides (mg/dL)	106 ± 52	95 ± 28	112 ± 62
Creatinine (mg/dL)	0.79 ± 0.2	0.74 ± 0.2	0.81 ± 0.2
Albumin (g/L)	34 ± 6.7	34 ± 7	34 ± 7
Bilirubin (mg/dL), Median (IQR)	1.3 (1.6)	1.5 (2.5)	1.2 (1.4)
ALT (U/L)	66 ± 54	57 ± 48	72 ± 58
Leucocytes (x10^9/L)	5.0 ± 2.1	5.4 ± 2.1	4.8 ± 2.0
Platelets (x10^{12}/L)	97 ± 40	109 ± 40	89 ± 39
Haemoglobin (g/L)	118 ± 26	114 ± 28	121 ± 24
Prothrombin activity (%)	67 ± 16	69 ± 14	66 ± 17

Results as Mean ± standard deviation if not indicated.
[a]$P < 0.05$.

TABLE 2 Hepatic haemodynamics of the patients and response to iv propranolol

	Overall (N = 66)	Non-responders (n = 25)	Responders (n = 41)
Baseline			
WHVP (mm Hg)	26.5 ± 5.5	27 ± 6	26 ± 5.5
FHVP (mm Hg)	9.5 ± 4	11 ± 4	8.5 ± 4.5[a]
HVPG (mm Hg)	17 ± 4	16 ± 3.5	17.5 ± 4
After iv propranolol			
WHVP (mm Hg)	25 ± 5.5	26.5 ± 6	24.5 ± 5.5
FHVP (mm Hg)	10.5 ± 4	10.5 ± 3.5	10.5 ± 4
HVPG (mm Hg)	14.5 ± 4	16 ± 3.5	14 ± 4.5[a]
Mean HVPG decrease (%)	13.9 ± 13.9	1.8 ± 5.5	21.3 ± 12.1[a]

Results as Mean ± standard deviation.
[a]$P < 0.05$ Resp vs Non-Resp.

3.1.1 | Metabolomic analysis and metabolite predictive models for HVPG response

A total of 389 metabolic features were identified at metabolomic broad profiling analysis. Of these 389 metabolites, 177 were selected for analysis according to their baseline chromatographic resolution criterion. Several metabolites were at different concentrations between responders and non-responders and most of them belonged to the chemical group of glycerophospholipids (also known as plasmalogens) and non-esterified fatty acids (NEFA), which were at higher concentrations in responders. Of these 177 metabolites, 18 were at different concentrations ($P \le 0.05$) between responders and

non-responders and were included for logistic regression analysis. These individual different metabolites are represented in Figure 1.

Logistic regression to develop a prognostic model for HVPG response was performed with combinations of two metabolites from the 18 finally selected. Several combinations of these metabolites showed a good performance (AUROC around 0.8), most of them including the NEFA 20:2(n-6) (eicosadienoic acid) (Table 3). Adding a third metabolite did not significantly improve the performance of these models. The finally selected model, the best in terms of AUROC and AIC, was composed by a phosphatidylcholine (and eicosadienoic acid). Coefficients of the model to calculate the probability of being responder were: α (intercept) = −3.179; β_1(Eicosadienoic) = 1.526; β_2(PC(P-16:0/22:6)) = 0.481. This model had a good discrimination with an AUROC of 0.80 (CI95% 0.688 − 0.914; Figure 2), a sensitivity of 85.4%, specificity of 60% and an AIC of 72.891. At internal leave-one-out cross-validation the model was shown to be robust with an AUC of 0.761 (Figure 2). Similar results were also obtained with the other potential combinations of metabolites (Table 3).

3.1.2 | Association of selected metabolites with clinical variables: primary/secondary prophylaxis, Child-Pugh class, aetiology and concomitant medications

The 18 metabolites associated with the HVPG response to propranolol were further analysed for association with a priori clinically relevant variables: type of prophylaxis, Child-Pugh class, aetiology and concomitant medications. Secondary prophylaxis was associated with a worse response (Table 1) but when added this variable to the metabolite model, it was not longer associated to response and did not modify metabolites predictions or coefficients.

Child class (B/C vs A) was associated with decreased levels of glycerophospholipids (except for (P-16:0/18:2)) and of free fatty acids (18:0) and (17:0). Therefore, despite Child was not associated to HVPG response, its potential effect on the model was explored. In this model, metabolites remained independent predictors of HVPG response, while Child did not (P = 0.210). The performance of the model including Child was similar (AUC 0.815, Se 82.9%, Sp 64%)

and AIC was not better than metabolite model (73.2 vs 72.9 respectively). Regarding other clinical variables, no modifying effects on metabolites were found among aetiologies (viral vs alcohol vs others) and predefined group of medications. Table S1 shows the effects of Child, prophylaxis and aetiology on metabolite model's coefficients and performance.

3.1.3 | Applicability of the metabolomic model: 1 and 2 cut-offs approaches

Cut-offs values to classify patients according to the HVPG response were studied with the metabolite model. With a unique cut-off approach, the cut-off value of 0.629, with a Youden index of 1.476, was selected based on a better balancing between misclassified responders and non-responders (Figure 3A). This value cut-off correctly classified 49 of the 66 patients (74.2% accuracy), adequately identifying 31/41 responders and 18/25 non-responders with seven false positives and 10 false negatives. This cut-off had 76% specificity, 72% sensitivity, 82% PPV and 64% NPV.

A two cut-offs approach was explored in order to minimize the number of misclassified patients but at expenses of creating an intermediate "grey zone" (Figure 3B). Two values optimizing positive and negative predictive values were proposed: 0.688 and 0.384 respectively. The first cut-off set at 0.688, with a PPV of 84%, allowed the identification of 27 of 41 responders with five false positives. The second value, set at 0.384 and with a NPV of 82%, allowed the identification of 14/25 non-responders with 3 false negatives. Between these two values, 17 patients would remain as unclassified (grey zone) and would require further haemodynamic study.

4 | DISCUSSION

In this pilot study, we provide a simple predictive model to identify HVPG responders to acute iv propranolol based on metabolomic serum analysis. The current study reveals several lipid substances at significantly different concentrations between HVPG responders and non-responders, most of them non-esterified fatty acids and glycerophospholipids (plasmalogens). Several combinations of these metabolites showed a good discrimination for HVPG response. In

FIGURE 1 Heatmap of individual metabolites at different concentrations between responders and non-responders. Most of these metabolites were NEFA and glycerophospholipids (plasmalogens), which were at higher concentrations in responders
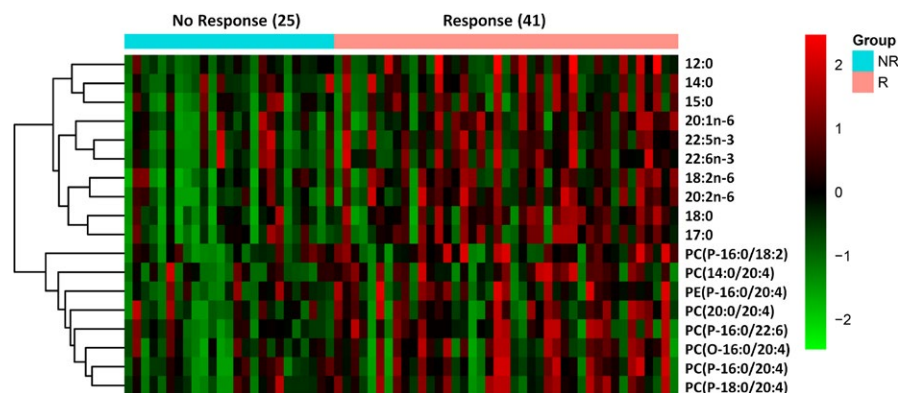
TABLE 3 Best metabolite prognostic models and their performance to predict the acute HVPG response to propranolol

Metabolites	Akaike IC	AUROC	Validation AUROC	Cut-off	Sens/Spec PPV/NPV (%)
Eicosadienoic acid (20:2(n-6)) + PC(P-16:0/22:6)	72.9	0.801 (0.69-0.91)	0.761 (0.63-0.89)	0.629	72/76 82/64
Eicosadienoic acid (20:2(n-6)) + PC(O-16:0/20:4)	74.3	0.799 (0.69-0.91)	0.762 (0.64-0.88)	0.543	81/64 79/67
Eicosadienoic acid (20:2(n-6)) + PC(P-18:0/20:4)	75.6	0.794 (0.68-0.91)	0.740 (0.61-0.87)	0.658	68/80 84/62
Gadoleic acid (20:1n-6) + PC(O-16:0/20:4)	77.1	0.791 (0.68-0.91)	0.745 (0.62-0.87)	0.688	66/88 90/61

Cut-off selection was done based on Youden Index and a good balancing between positive/negative misclassification.
NPV, negative predictive value; PPV, Positive predictive value.
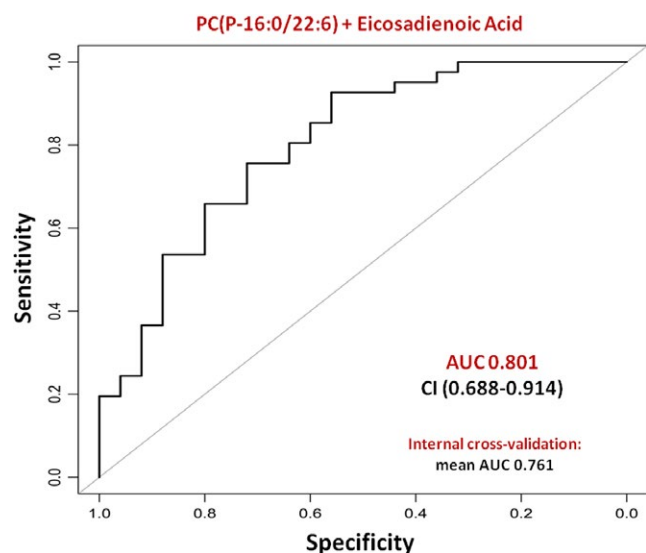
FIGURE 2 Performance of the model including eicosadienoic acid (20:2(n-6)) and the plasmalogen PC(P-16:0/22:6) to predict the acute response of HVPG to propranolol

agreement with previous studies,[2,3,5,6,12,14,28,29] no association between HVPG response and baseline variables, except for primary/secondary prophylaxis patients was observed. However, this variable (or any clinical variable) did not add to the prediction of the metabolite model.

In a pragmatic approach, we decided to evaluate the model including a plasmalogen (PC(P-16:0/22:6)) and eicosadienoic acid, which showed a slightly (despite non-significant) better AUROC curve (AUROC 0.801). Using the Youden approach, 0.629 was the best cut-off value with a good overall performance maintaining a similar proportion of responders/non-responders well classified and misclassified (Figure 3A). However, from a clinical perspective, misclassifying non-responders as responders may prevent these patients to be shifted to more effective (usually also more invasive) therapeutic alternatives. By contrast, responders who would be misclassified as non-responders could be "overtreated" and potentially exposed to therapeutic secondary effects. Taking all these considerations in mind, we decided to also propose a two cut-off approach with a higher capacity to identify HVPG

responders and non-responders. With this approach, 0.688 would be a useful upper cut-off value identifying 27 of the 41 responders, with only five false-positive patients. The selection of a lower cut-off of 0.384 allowed the identification of 14 of the 25 non-responders with only three false negatives (Figure 3B). Thus, this two cut-off approach would allow the correct classification of 72% responders and 60% non-responders. However, 17 patients (25% of our cohort) had values among these two cut-off being therefore not-classified (grey zone) requiring the HVPG study. The use of this strategy may be useful, for example, in scenarios like clinical trials assessing the role of more aggressive treatments, such as TIPS, in HVPG non-responders: patients with values above 0.688 (mostly responders) would not be included, those below 0.384 (mostly non-responders) could be directly included. Patients falling in the "grey zone" (one-quarter of the cohort), would require the HVPG study. However, it would be possible to avoid the remaining 75% HVPG studies (Figure 3B). This dual approach would also be interesting for centres with limited availability for HVPG measurements. There were many other significantly different metabolites according to response and our model was finally selected from a data-driven analysis though probably other potential models could also be useful (Table 2). The present model may offer advantages over previous described non-invasive methods to assess HVPG response, mainly changes in femoral/portal flow. Our model would only require peripheral blood extraction, while other techniques require expertise and time (measures before and after NSBB) and associate individual and explorer variability. Therefore, the metabolite approach would seem a reasonable non-invasive technique for HVPG response assessment.

A thorough study of potential factors influencing the selected metabolites beyond HVPG was done and a lower concentration of glycerophospholipids was found in advanced Child-Pugh class. Among the selected metabolites to develop the model, no association with aetiology (alcohol, viral and others) or concomitant medications was found. The association of glycerophospholipids with Child-Pugh was independent of the HVPG response and the inclusion of Child to the metabolite model did not significantly improve its predictions: similar AUC, AIC and misclassification rate. Even more, Child-Pugh was not even associated with HVPG response at initial

FIGURE 3 Applicability of the metabolomic model. A, One cut-off approach (totally non-invasive): The cut-off value of 0.629 allows the correct classification of 74% (49/66) of the patients; 17 patients are misclassified (seven false positives and 10 false negatives). B, Two cut-offs approach: 0.688 and 0.384 showed good positive and negative predictive values: 84% and 82% respectively. The cut-off 0.688 identified 27 of 41 responders (five false positives), while 0.384 identified 14/25 non-responders (three false negatives). Seventeen patients fell in the grey zone
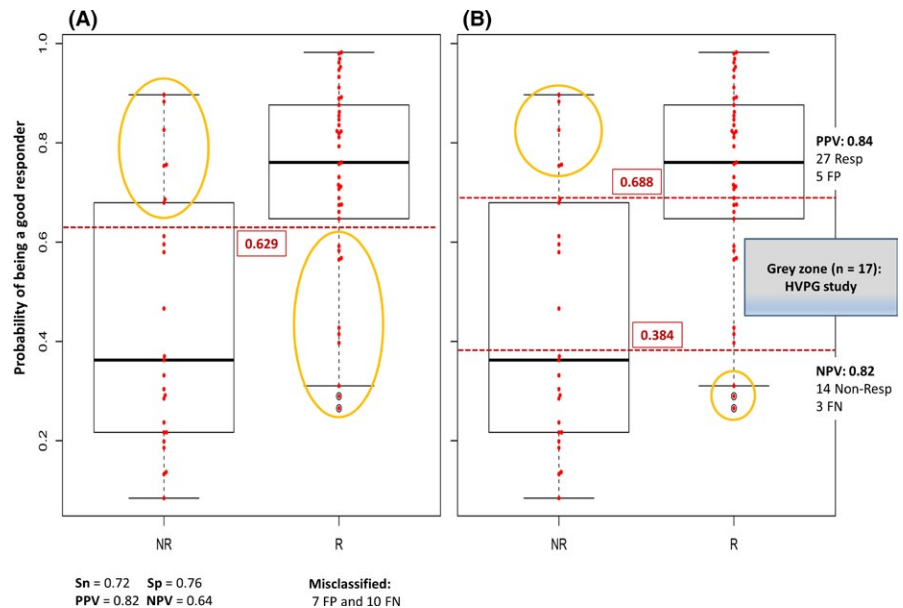
analysis. For all these reasons a purely metabolite prognostic model was finally selected. However, the potential effect of Child on these metabolites should be taken into account and it might even be better characterized in larger studies, where this effect might become relevant. Other analysed clinical variables did not add or modified the model.

The choice of metabolomic analysis over other high-throughput techniques (genomics, transcriptomics, proteomics) may be justified for several reasons, though probably none of them is superior but complementary. First of all, the experience of the group with studies on idiopathic PHT was promising and showed a good diagnostic accuracy for metabolomics. The advantage of studying metabolome is that reflects the last step of gene expression and in addition, it is also affected by internal and external factors (health status, age, diet, exercise, etc), which finally reflects a "global summary" of the patient. Observations from OWL metabolomics (not published) showed that metabolome and lipid analysis are homogenous and reproducible along the time in fasting conditions. Finally, in a targeted metabolomic analysis like this, once the metabolite is identified, a diagnostic test including these substances can be easily developed. However, it must be recognized that an integrative approach of metabolomics with other high-throughput techniques might throw more precise predictions.

We decided to work with the acute HVPG test instead of the chronic HVPG response to propranolol because of its applicability: it is easier for patients to undergo one study and the 10% cut-off has shown to effectively detect patients at lower risk of bleeding and decompensation.[5,6] However, it must be acknowledged that the study of chronic response could have a higher specificity to detect non-responders. Whether the different metabolites observed in HVPG responders and non-responders are reflecting different pathophysiological mechanisms involved in response to β-blockers or are just non-invasive markers remains unclear. Most of the different metabolites between responders/non-responders considered for prognostic models were NEFA and glycerophospholipids

(plasmalogens), both at lower concentrations in non-responders. NEFA levels are increased by norepinephrine in hyperadrenergic and water retentive states such as in cardiac failure[30] as well as in cirrhosis.[31,32] This effect is mediated through β-adrenoceptors and the hormone-sensitive lipase.[33,34] The fact that non-responders showed lower NEFA levels, may reflect a lesser β-adrenergic stimulation or a metabolic resistance of the β-adrenoceptor that might explain that NSBB did not reach the desired effect. Glycerophospholipids have been proposed as protective agents in animal models of NASH and declining levels have been described in parallel to liver fibrosis progression.[35,36] Therefore, it might be hypothesized that lower levels of glycerophospholids observed in HVPG non-responders might be related to a more fibrogenic phenotype of cirrhosis, which could be less dependent and modifiable by vasoactive systems such as β-adrenergic stimulation/blockade.

The present study has some limitations. Despite a robust internal cross-validation, our model has not been externally validated. The cross-sectional nature of this study did not include follow-up serial measurements (neither metabolites nor HVPG), so a prognostic role for metabolites beyond response cannot be elucidated. This cross-sectional nature also limits mechanistic pathophysiological interpretations regarding the relationship between metabolites and HVPG response. Finally, our study included different type of patients regarding aetiology, Child and prophylaxis type, which may affect the metabolites profile and introduce undetected bias. A thorough analysis of these factors found an association of metabolites with Child-Pugh though it did not modify the metabolite model. It is possible that in larger series Child-Pugh may become and adjustment variable and improves predictions. Less prevalent comorbidities in our cohort (diabetes, dyslipidemia, arterial hypertension) or external factors (exercise, diet) may also account for uncontrolled bias or influence in metabolites.

In conclusion, the combination of two serum metabolites might help at identifying the HVPG response to acute iv propranolol in

patients with cirrhosis. The analysis of these metabolites could be a useful non-invasive tool to identify these patients though further validation of the model would be desirable.

CONFLICT OF INTEREST

The authors disclose no conflicts.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.