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Impact of hepatopulmonary syndrome in liver transplantation candidates and the role of angiogenesis

Steven M. Kawut[1,2], **Michael J. Krowka**[3], **Kimberly A. Forde**[1,2], **Nadine Al-Naamani**[1], **Karen L. Krok**[4], **Mamta Patel**[1,2], **Carlo R. Bartoli**[5,6], **Margaret Doyle**[7], **Jude Moutchia**[2], **Grace Lin**[3], **Jae K. Oh**[3], **Carl D. Mottram**[3], **Paul D. Scanlon**[3], **Michael B. Fallon**[8], **Pulmonary Vascular Complications of Liver Disease Study Group**

[1]Dept of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

[2]Center for Clinical Epidemiology and Biostatistics, Dept of Biostatistics, Epidemiology, and Informatics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

[3]Dept of Medicine, Mayo Clinic, Rochester, MN, USA.

[4]Dept of Medicine, Penn State Milton S. Hershey Medical Center, Hershey, PA, USA.

[5]Division of Cardiovascular Surgery, Dept of Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

[6]Division of Cardiothoracic Surgery, Dept of Medicine, Geisinger Medical Center, Danville, PA, USA.

[7]Dept of Pathology, University of Vermont, Burlington, VT, USA.

[8]Dept of Medicine, University of Arizona, Phoenix, AZ, USA.

Abstract

Background—Hepatopulmonary syndrome affects 10–30% of patients with cirrhosis and portal hypertension. We evaluated the serum angiogenic profile of hepatopulmonary syndrome and assessed the clinical impact of hepatopulmonary syndrome in patients evaluated for liver transplantation.

Methods—The Pulmonary Vascular Complications of Liver Disease 2 study was a multicentre, prospective cohort study of adults undergoing their first liver transplantation evaluation. Hepatopulmonary syndrome was defined as an alveolar–arterial oxygen gradient ⩾15 mmHg (⩾20 mmHg if age >64 years), positive contrast-enhanced transthoracic echocardiography and absence of lung disease.

Results—We included 85 patients with hepatopulmonary syndrome and 146 patients without hepatopulmonary syndrome. Patients with hepatopulmonary syndrome had more complications of

Corresponding author: Steven M. Kawut (kawut@upenn.edu).

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portal hypertension and slightly higher Model for End-Stage Liver Disease-Na score compared to those without hepatopulmonary syndrome (median (interquartile range) 15 (12–19) *versus* 14 (10–17), p=0.006). Hepatopulmonary syndrome patients had significantly lower 6-min walk distance and worse functional class. Hepatopulmonary syndrome patients had higher circulating angiopoietin 2, Tie2, tenascin C, tyrosine protein kinase Kit (c-Kit), vascular cell adhesion molecule 1 and von Willebrand factor levels, and lower E-selectin levels. Patients with hepatopulmonary syndrome had an increased risk of death (hazard ratio 1.80, 95% CI 1.03–3.16, p=0.04), which persisted despite adjustment for covariates (hazard ratio 1.79, 95% CI 1.02–3.15, p=0.04). This association did not vary based on levels of oxygenation, reflecting the severity of hepatopulmonary syndrome.

Conclusion—Hepatopulmonary syndrome was associated with a profile of abnormal systemic angiogenesis, worse exercise and functional capacity, and an overall increased risk of death.

Shareable abstract (@ERSpublications)

The presence of hepatopulmonary syndrome with even mild oxygenation abnormalities was associated with shorter survival in candidates evaluated for liver transplantation and was characterised by higher levels of pro-angiogenic biomarkers https://bit.ly/3EAihft

Introduction

Hepatopulmonary syndrome (HPS) occurs when intrapulmonary vascular dilation and pulmonary arteriovenous malformations lead to abnormal systemic oxygenation in the setting of liver disease or portal hypertension [1]. This syndrome has been found in 10–30% of patients with cirrhosis being evaluated for liver transplantation [2–4]. We and others have shown that HPS is associated with a doubling in the risk of death even after accounting for liver transplantation, which is curative [3, 5, 6].

The mechanism of HPS is currently unknown. Prior studies of patients and the experimental model of HPS have suggested that dysregulated angiogenesis plays an important role in this manifestation of advanced liver disease. Increased circulating levels of haematopoietic progenitor cells/monocytes were found in the common bile duct ligation rat model [7–9]. Genetic variants of the gene that codes for von Willebrand factor (vWF) and higher vWF levels as well as the precursor of endostatin were associated with HPS in a prior study [10]. Anti-angiogenic interventions (*e.g.* endostatin and angiostatin expression and sorafenib) improved gas exchange and shunting in the HPS experimental model [11]. While a small phase II randomised double-blind placebo-controlled trial of sorafenib showed a reduction in vascular endothelial growth factor (VEGF) receptor-2, sorafenib did not affect gas exchange, exercise capacity or quality of life in patients with HPS [12].

Some have questioned the importance of the impact of HPS, especially when it presents subclinically without significant hypoxaemia. We previously published a prospective multicentre cohort of liver transplantation candidates in which most HPS was characterised by mild reductions in arterial oxygen tension (P_{aO2}) and oxygen saturation in arterial blood and infrequent severe hypoxaemia [3]. Even so, HPS had a clinically significant negative impact on functional status, health-related quality of life and survival. However, there are no

prospective, multicentre human studies of the angiogenic milieu of HPS and the impact of HPS on other clinical end-points with systematic evaluation of heart and lung function and adjustment for confounders.

Therefore, we evaluated the clinical characteristics, angiogenic biomarker profile, exercise capacity and risk of hospitalisation and mortality in patients with HPS compared to those without HPS with advanced liver disease being evaluated for liver transplantation.

Methods

Study design and study sample

The Pulmonary Vascular Complications of Liver Disease (PVCLD2) study enrolled a cohort of 454 patients evaluated for liver transplantation at centres in the USA between 2013 and 2017 (supplementary figure E1). The only inclusion criterion was the presence of portal hypertension with or without intrinsic liver disease. We excluded patients with active infection or recent (<2 weeks) gastrointestinal bleeding and patients who had undergone prior liver or lung transplantation. The study was approved by the institutional review board of each centre.

The study sample for this analysis was drawn from enrolled patients undergoing their first liver transplantation evaluation at the University of Pennsylvania, Mayo Clinic and the University of Texas at Houston. We excluded patients thought to have portopulmonary hypertension.

HPS was defined as 1) alveolar–arterial oxygen tension difference (P_{A-aO2}) ⩾ 15 mmHg (or P_{A-aO2} ⩾ 20 mmHg if age was >64 years), 2) late passage of contrast on contrast-enhanced transthoracic echocardiography (positive contrast-enhanced transthoracic echocardiography), 3) absence of a significant obstructive and restrictive ventilatory defect on spirometry and 4) absence of intracardiac shunting [1, 3].

The main analysis excluded patients with obstructive or restrictive ventilatory defects (defined below), missing testing and those with intracardiac shunting from the control group. A sensitivity analysis for outcomes included all other patients undergoing their first liver transplantation evaluation without portopulmonary hypertension who did not meet diagnostic criteria for HPS.

Data collection and variables

Informed consent was obtained from eligible patients, who were then scheduled for research assessment, which included medical history, anthropometrics, physical examination, phlebotomy, pulse oximetry, arterial blood gas sampling, spirometry, 6-min walk testing and contrast echocardiography. All study visits and study procedures were conducted in the outpatient setting. Patients were asked to avoid smoking before the research assessment.

Phlebotomy was performed after overnight fasting except water. Serum and plasma were banked at −80°C, while samples for flow cytometry were processed within 24 h. All samples were shipped and assays performed at the University of Vermont Laboratory for Clinical

Biochemistry Research except for vWF multimer studies, which were performed at the University of Pennsylvania.

Clinical data were collected from formal interviews on the date of study procedures and from the medical record. Pulse oximetry was performed using a standard professional grade oximeter after the study participant maintained an upright seated posture for 5 min and was then repositioned supine for 5 min. Patients underwent a physical examination. Clinical laboratory results obtained closest to the date of the study visit were recorded. The Model for End-Stage Liver Disease (MELD-Na) score was calculated using the following formulae: MELD=10×((0.957×ln(creatinine))+(0.378×ln(bilirubin))+(1.12×ln(international normalised ratio)))+0.643 and MELD-Na=MELD+(1.32×(137−Na))−(0.033×MELD×(137−Na)) [13, 14].

Radial artery blood gas sampling was performed on ambient air in a seated position after 10 min of rest. The samples were processed in a blood gas analyser after a one-point calibration. The P_{A-aO_2} was calculated using the following formula: $P_{A-aO_2} = [(F_{iO_2} \times [P_{atm} - P_{H_2O}]) - (P_{aCO_2}/R)]] - P_{aO_2}$ where F_{iO_2} was the inspiratory oxygen fraction, P_{atm} was the barometric pressure measured on the date (and in the city) of the study visit, P_{aCO_2} was the arterial carbon dioxide tension and R was assumed to be 0.8 [15].

Pre-bronchodilator spirometry was performed according to American Thoracic Society and European Respiratory Society recommendations [16]. Obstructive ventilatory defect was defined as forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC)<0.70 with FEV_1<80% predicted, and restrictive ventilatory defect was defined as FVC<70% predicted. A minimum of three efforts with no acceptability errors and at least two with repeatability per standards (FVC within 150 mL of largest, FEV_1 within 150 mL of largest and peak flow within 15% of largest) were required. Testing was continued until the above criteria were met, a total of eight tests were performed or the patient was unable to continue testing. Sex-, age- and race-specific equations were used to determine per cent predicted based on spirometric reference values derived from the National Health and Nutrition Evaluation Survey III [17]. The 6-min walk test was performed according to American Thoracic Society guidelines [18].

Contrast-enhanced transthoracic echocardiography was performed by injecting agitated saline *via* a peripheral vein during transthoracic echocardiographic imaging. The apical four-chamber view was the preferred window for image acquisition, although the parasternal long axis, modified or para-apical four-chamber view, or subcostal views were utilised if the four-chamber view was suboptimal or unavailable. At least 10 continuous cardiac cycles were captured, beginning immediately prior to contrast injection so that cardiac cycles could be accurately assessed to determine the delay from the injection of agitated saline to visualisation of contrast entering the left heart. Identification of microbubbles in either the left atrium or left ventricle after three or more cardiac cycles was considered to indicate the presence of intrapulmonary vascular dilatation [19]. Patients with evidence of immediate (less than three cycles) opacification of the left atrium or left ventricle were presumed to have an intracardiac shunt. A Doppler flow signal across the atrial septum was presumed to indicate a patent foramen ovale, also considered to be an intracardiac shunt. Post-Valsalva

images were not utilised for study purposes. The Echocardiography Core Laboratory at the Mayo Clinic evaluated all contrast echocardiograms performed at individual study sites and echocardiographers interpreted the studies offline while blinded to all clinical information.

World Health Organization functional class

Assessment of symptoms and World Health Organization functional class was performed at baseline. The World Health Organization functional classification is modified from the New York Heart Association functional classification, with Class I defined as no symptoms, Class II as symptoms with more than usual activity, Class III as symptoms with less than usual activity and Class IV as symptoms at rest.

Patients were contacted by the research team every 6 months until 2017. Dates of hospitalisation, liver transplantation and death were obtained from the patients, medical records and the patients' physicians. Patients who were alive at the end of follow-up were censored at May 2017.

Laboratory assays

All assays were performed in bulk at the conclusion of the study except for flow cytometry. We used the MILLIPLEX Human Angiogenesis Panel 2 (#HANG2MAG-12K), which is a bead-based Luminex multiplex assay, to measure plasma angiostatin, soluble tyrosine protein kinase Kit (c-Kit), soluble E-selectin, soluble epithelial growth factor receptor, tenascin C, soluble Tie2, soluble VEGF receptors-1, −2 and −3, platelet-derived growth factor AB/BB and platelet endothelial cell adhesion molecule-1 (MilliporeSigma, Burlington, MA, USA). We used the MILLIPLEX Human Cytokine/Chemokine Magnetic Bead Panel - Immunology Multiplex Assay (#HCYTOMAG-60K) to measure plasma fractalkine and VEGFA (MilliporeSigma). We measured plasma angiopoietin 2 using the Meso Scale Discovery Human Angiopoietin-2 Kit (#K151KCD, Mesa Scale Diagnostics, Rockville, MD, USA). We measured plasma vascular cell adhesion molecule 1 (#DVC00) and plasma endostatin (#DNST0) using ELISAs (R&D Systems, Minneapolis, MN, USA). vWF antigen was assessed using an immunoturbidimetric method from Stago (cat #00518).

Details of vWF multimer studies and flow cytometry are provided in the supplementary material. Because flow cytometry occurred in "real time" throughout the cohort, we used the standard flow cytometric classifications at the time of cohort initiation, which have generally remained similar [20]. We focused on haematopoietic progenitor cells defined by $CD34^+$, $CD34^+CD133^+$ and $CD34^+CD133^+KDR^+$, which were CD45dim (expressed as % of peripheral blood mononuclear cells) and intermediate class (M2) monocytes and Tie2-expressing M2 monocytes ($CD14^+CD16^+$ and $CD14^+CD16^+Tie2^+$), both expressed as percentage of $CD14^+$ cells.

Statistical analysis

Continuous data were summarised using mean±SD or median (interquartile range (IQR)), as appropriate. Categorical variables were summarised with n (%). We compared HPS to non-HPS patients using unpaired t-tests, Wilcoxon rank sum tests, chi-squared tests and Fisher's exact tests, as appropriate. We used bivariate and multivariate linear regression to

analyse the association between HPS status and the 6-min walk distance and biomarkers of angiogenesis.

We analysed the association of HPS with the risk of hospitalisation using relevant models for recurrent event analysis including gamma frailty, Andersen–Gill, Prentice–Williams–Peterson gap-time and total-time, and multistate models [21]. Survival was assessed using the Kaplan–Meier estimator and Cox proportional hazards models and expressed with a hazard ratio (HR) in bivariate and multivariate analyses. We included age and MELD-Na as covariates in the multivariable models. We analysed overall survival (including time before and after liver transplantation) as the primary analysis, because ultimately that is what is of clinical importance to patients and clinicians. We performed sensitivity analyses with censoring at liver transplantation, considering liver transplantation a competing risk for death [22], and adjusting for liver transplantation as a time-varying covariate. We also performed multistate modelling [23]. We calculated E-values for the main survival analyses [24]. We used laboratory parameters that were correlated with MELD-Na and a random forest imputation algorithm to impute missing MELD-Na scores (2%) for the adjusted analyses [25]. Owing to the independent hypotheses investigated, there was no correction for multiple comparisons. All analyses used R version 3.6.1 (www.r-project.com).

Results

A total of 454 patients were enrolled in the cohort (supplementary figure E1). 43 were excluded for presumed portopulmonary hypertension, leaving 411. Of these, 26 were excluded for lack of arterial blood gas or pulmonary function testing, 40 were excluded for obstructive (and 65 for restrictive) ventilatory defects and 49 had evidence of patent foramina ovalia. Of the remaining 231, 85 (37%, 95% CI 31– 43%) met criteria for HPS. Of the full cohort without a possible diagnosis of portopulmonary hypertension (n=411), at least 21% (95% CI 17–25%) had HPS. There were no substantive differences between the final study sample (n=231) and those new patients without possible portopulmonary hypertension who were excluded (n=180) (supplementary table E1).

There were 85 patients with HPS and 146 without HPS (table 1). Patients with HPS were slightly younger and more likely to be female. HPS patients were more likely to be non-Hispanic white than non-HPS patients, but had similar educational attainment and household income. A high proportion of patients in both groups had liver disease attributable to alcohol use and/or hepatitis C infection. The median MELD-Na score was one point higher in patients with HPS compared to those without HPS (15, IQR 12–19, versus 14, 10–17; p=0.006). Patients with HPS were more likely to have a history of ascites, varices and encephalopathy but were less likely to have a history of hepatocellular carcinoma. Smoking and alcohol use were similar between the groups.

Dyspnoea was more common and functional class was significantly worse in patients with HPS (table 2); cyanosis and jaundice were more common in HPS. Other physical examination findings such as clubbing and asterixis appeared to be more common in patients with HPS although ascites, spider angiomata and an increased degree of encephalopathy were not.

Patients with HPS had a mean oxygen saturation of 96% by pulse oximetry while sitting which was on average only 2% lower than the oxygen saturation of liver disease patients without HPS (table 2). While orthodeoxia (decrease in oxygen saturation by pulse oximetry ⩾3% from supine to seated position) was significantly more common in HPS only 12% of patients with HPS demonstrated this. Lung function between the groups was similar and P_{aO_2} and P_{A-aO_2} were significantly different between the groups (table 2). Only seven HPS patients (8%) had a $P_{aO_2} < 60$ mmHg or oxygen saturation by pulse oximetry <90% on ambient air. Abdominal imaging demonstrated ascites in 50 patients with HPS (60.2%) and 67 patients without HPS (46.5%) (p=0.05). Six patients in each group had portal vein thrombosis (p=0.36).

Patients with HPS had a 29 m (95% CI 3–56 m) shorter 6-min walk distance compared to liver disease controls with adjustment for age, sex and MELD-Na (p=0.04, n=197) (figure 1). There were no significant differences in oxygen saturation, heart rate or Borg score at the end of the walk.

We performed a panel of blood biomarkers of angiogenesis with adjustment for age and MELD-Na score (figure 2 and supplementary table E2). Several pro-angiogenic biomarkers were significantly higher in patients with HPS compared to liver disease controls including angiopoietin 2, c-Kit, vascular cell adhesion molecule 1 and tenascin C. Tie2 and platelet-derived growth factor showed a nonsignificant increase in HPS patients compared to liver disease controls. Endostatin and angiostatin (both anti-angiogenic molecules) showed a nonsignificant reduction in HPS. We did not find differences in VEGF1 or VEGF receptors or other protein or flow cytometry biomarkers (supplementary table E2). There was no association between angiogenesis biomarkers and P_{aO2} or P_{A-aO_2} after adjustment for MELD-Na in patients with HPS (data not shown).

vWF antigen levels were significantly higher in patients with HPS compared to liver disease controls (figure 3 and supplementary table E2). Based on this finding, we performed analyses of circulating vWF multimer size in 40 randomly selected patients with HPS and 60 liver disease controls. HPS patients had significantly higher circulating levels of low-molecular-weight vWF multimers and vWF degradation fragments compared to liver disease controls after adjustment for age and MELD-Na. These findings were accompanied by significantly elevated levels of vWF clotting function in HPS (vWF:collagen binding). Levels of vWF antigen were strongly associated with angiopoietin 2 levels (r=0.50, p<0.001).

The median follow-up time in the cohort was 2 years (IQR 1.2–2.8 years), and there were 461.1 person-years of follow-up. 92 patients underwent liver transplantation (~40% in each group), and there was no difference in the time to liver transplantation (supplementary figure E2). 13% of patients were not censored as alive or dead by the end of follow-up.

There were 421 hospitalisations (89 per 100 person-years); the median number of hospitalisations per patient was one (IQR 0–3). Liver transplantation was not considered as a hospitalisation. Supplementary figure E3 shows that the mean cumulative function plots of hospitalisations were similar for patients with HPS and liver disease controls,

and supplementary figure E4 shows the number of hospitalisations in the groups. HPS was not associated with the risk of recurrent hospitalisation with adjustment for age, sex and MELD-Na using the gamma frailty model (HR 1.06, 95% CI 0.77–1.46, p=0.70). Sensitivity analyses with adjustment for transplantation, examining transplantation-free hospitalisation, and using Andersen–Gill, Prentice–Williams–Peterson gap-time and total-time, and multistate models still showed no difference in hospitalisation (data not shown).

Patients with HPS had worse survival than liver disease controls (figure 4, log rank test p=0.04). 24 HPS patients (28%) and 25 liver disease controls (17%) died during follow-up. Patients with HPS had a lower probability of being alive than liver disease controls at 1 year (87% *versus* 92%), 2 years (73% *versus* 83%) and 3 years (63% *versus* 81%). Causes of death are shown in supplementary table E3. Cox proportional hazards models showed that patients with HPS had an 80% increase in the risk of death in bivariate (HR 1.80, 95% CI 1.03–3.16, p=0.04) and multivariate (HR 1.79, 95% CI 1.02–3.15, p=0.04) analyses adjusted for age and MELD-Na (table 3). A model with adjustment for liver transplantation as a time-varying covariate showed similar results (table 3). When transplantation was considered as a competing risk using the Fine–Gray model, the sub-distributional HR of HPS *versus* liver disease controls for death was 1.91 (95% CI 1.06–3.45, p=0.03) after adjustment for age and MELD-Na (figure 5). A multistate model (supplementary figure E5) suggested that HPS was associated with an increased risk of death in patients without liver transplantation (HR 1.84, 95% CI 0.99–3.44, p=0.05) but not with the chances of receiving liver transplantation (HR 1.03, 95% CI 0.67–1.58, p=0.89) or the risk of death after liver transplantation (HR 0.99, 95% CI 0.24–4.15, p=0.99).

The association of HPS with overall survival was partially attenuated after adjustment for angiopoietin 2 (28% attenuated, HPS *versus* no HPS HR=1.30, 95% CI 0.70–2.41, p=0.40) or vWF levels (21% attenuated, HPS *versus* no HPS HR=1.42, 95% CI 0.77–2.61, p=0.30). This suggests that these biomarkers were in the causal pathway or were confounders of the association of HPS with outcomes. Other biomarkers did not have qualitatively important impacts on the effect estimate. After excluding patients with hepatocellular carcinoma, findings were generally consistent with the main results albeit nonsignificant in some cases with the smaller sample size and lower power.

The differences in overall risk of death for HPS *versus* liver disease controls did not differ based on P_{aO2} (p for interaction=0.30) or P_{A-aO_2} (p for interaction=0.40), suggesting that the relationship between HPS and worse outcomes was not dependent on the severity of HPS. We also compared the survival of patients with HPS with all others in the prospective cohort without portopulmonary hypertension, including those with restrictive or obstructive lung diseases, patent foramina ovalia, or missing data who were excluded from the primary analyses. Many of these excluded patients likely had HPS coexisting with their underlying exclusion. For example, of the 105 patients excluded for restrictive or obstructive lung disease, 49 (47%) had a positive contrast-enhanced transthoracic echo and 38 (36%) had abnormal P_{A-aO_2} and a positive contrast-enhanced transthoracic echo. Even with including these patients in the “control” group, HPS still appeared to be associated with a higher risk

of death (HR 1.56, 95% CI 0.97–2.50, p=0.07) with a weaker effect estimate, as expected with the likely presence of undiagnosable HPS in the “control” group.

Discussion

We have shown that HPS was a common complication in patients who were referred for evaluation for liver transplantation. In contrast to some prior studies, patients with HPS had more severe liver disease and complications of portal hypertension. Despite only mild abnormalities in oxygenation of the blood, with only 8% being clinically hypoxaemic, HPS patients had more respiratory symptoms, worse functional class and lower 6-min walk distance after adjustment for severity of liver disease, which is a novel finding. HPS patients were characterised by a profile of dysregulated angiogenic peptides compared to liver disease controls even after accounting for differences in the severity of liver disease, which has not been demonstrated previously. Patients with HPS had a risk of hospitalisation that was similar to that of liver disease controls; however, HPS patients had a significantly increased risk of death overall regardless of the degree of abnormal oxygenation. Some of this increased risk was accounted for by higher levels of angiopoietin 2 and vWF in patients with HPS, representing the first possible biological mechanisms for how HPS affects outcome in patients. This study used sophisticated research-grade prospective heart and lung phenotyping and adjusted for confounders in multivariate analyses (notably severity of liver disease), distinguishing our results from those of other studies.

We found that 37% of our patients without other potential causes of oxygen abnormalities and ⩾21% of the entire cohort had HPS based on established diagnostic criteria. A prior study (which recruited candidates for liver transplantation 10 years before the current study) did not show differences in severity of liver disease between patients with HPS and those without HPS [3]. This may be due to the evolving characteristics of patients being evaluated for liver transplantation over time or spectrum bias. For example, almost one quarter of patients in the current sample had non-alcoholic fatty liver disease compared to only 11% in the prior study, and approximately one third of the current sample had hepatocellular carcinoma compared to only 9% in the prior study. These differences may reflect not only changes in the general population (*e.g.* increased obesity) and aetiologies of advanced liver disease but also newer exception point policies that prioritise certain patients for transplantation (*e.g.* with hepatocellular carcinoma) in the USA, thereby influencing the composition of referrals for evaluation. The higher severity of liver disease in patients with HPS in the current study (reflected by higher MELD-Na score and more complications of portal hypertension) could either be a cause or consequence of HPS and has been seen in other recent cohorts [2, 4, 26]. One study of patients listed for liver transplantation actually showed lower laboratory MELD scores in those with HPS listed for liver transplantation (although this study’s focus on HPS with exception scores could introduce selection bias) [27]. More severe liver disease could lead to HPS as one of the sequelae or the presence of HPS could causally contribute to more liver disease complications; angiogenesis or other pathobiological processes could cause both worse liver disease and HPS.

There were small differences in oxygen saturation in HPS and non-HPS patients, and only 8% of HPS patients were hypoxaemic [3]. Even so, patients with HPS had both clinically

and statistically significantly shorter distance walked in 6 min compared to other patients with liver disease. This difference in exercise capacity substantiates the greater symptoms reported by patients with HPS and the worse functional class assigned by clinicians [28]. Worse liver disease did not explain the difference in exercise capacity because this was independent of MELD-Na. Other systemic processes may cause worse symptoms and exercise capacity in HPS.

Studies in experimental models of HPS have demonstrated increased lung angiogenesis [11]. We showed higher levels of several important circulating biomarkers of angiogenesis, including angiopoietin 2, c-Kit and possibly Tie2, in HPS even after adjustment for the differences in severity of liver disease. Higher angiopoietin 2 (which signals *via* Tie2) has been linked to increased pathological angiogenesis in several studies of patients with liver disease [29–31]. Tenascin C is an extracellular matrix protein that has been linked to vascular remodelling in the lung. Both angiostatin and endostatin tended to be lower in HPS, which parallels their roles as anti-angiogenic molecules preventing HPS in the experimental model [11]. Vascular cell adhesion molecule 1 was higher in HPS as in one prior study, suggesting that leukocyte adhesion to the endothelium may play a role [32]. We have previously demonstrated lower bone morphogenetic protein 9 and 10 levels (linked to increased angiogenesis) in a small sample of HPS patients from this cohort [33].

HPS patients had significantly higher levels of vWF antigen, low-molecular-weight vWF multimers and vWF degradation fragments. Levels of vWF multimers and/or vWF degradation fragments may alter angiopoietin signalling, a potent destabiliser of blood vessels [34–36]; vWF levels were significantly associated with angiopoietin 2 levels in our study [34–38]. Indeed, abnormalities in vWF metabolism are associated with angiodysplasia and arteriovascular malformations in multiple diseases [39–46]. In infants with single ventricle anatomy and a superior-cavopulmonary (Glenn) circulation, abnormalities in vWF and angiopoietin 2 play a role in the development of pulmonary angiodysplasia and arteriovascular malformations also without increased VEGF [47]. High shear stress from continuous-flow left ventricular assist devices increases enzymatic degradation of large vWF multimers into vWF degradation fragments [37], which may alter circulating levels of angiopoietin 2 [48] and cause mucosal arteriovascular malformations [37, 38]. Patients with HPS may more commonly have varices, which may relate to these biomarker findings.

There was no association between the presence of HPS and the risk of hospitalisation. Even so, we found a significant association between HPS and a higher overall risk of death with similar findings when adjusting for liver transplantation, analysing transplantation-free survival and with liver transplantation as a competing risk. The major population accounting for this finding appeared to be patients who did not receive liver transplantation; HPS does not generally pose a significant increase in risk after liver transplantation in recent studies [2, 26, 27, 49]. When comparing patients with HPS to the rest of the liver transplantation candidates in our cohort (including patients with restrictive and obstructive lung diseases, patent foramina ovalia, etc.), there was still a 56% increase in risk of death overall (95% CI −3% to 250%), a conservative estimate considering that the comparison group likely included up to one third of patients with HPS (undiagnosable due to the other comorbid conditions).

Prior studies of survival have been single centre, without multivariate analyses, and without careful phenotyping of the presence of HPS (or the exclusion of patients with other reasons for abnormal oxygenation) [4–6, 50]. Our prior prospective multicentre cohort study showed that HPS was independently associated with an increased risk of death even after adjusting for MELD score and liver transplantation [3]. More recently, two single-centre cohort studies showed that HPS was associated with an increased risk of death, but not after multivariate adjustment [4, 26]. In a prior study of patients listed for liver transplantation in the United Network for Organ Sharing, we found that patients with HPS actually had better overall survival than patients without HPS, which was attributable to HPS patients receiving higher priority for liver transplantation with MELD exception [27].

The association of HPS with worse outcomes in liver disease has vexed some owing to the very mild subclinical abnormalities in oxygenation in most HPS patients. However, we have now replicated this finding in two distinct multicentre prospective cohort studies with similar effect estimates. The role of angiogenesis (which causes other well-known sequelae of portal hypertension) may address the question of how HPS affects survival, although findings from a recent clinical trial of sorafenib to target angiogenesis in patient with HPS were null [12].

The only therapy that reverses HPS is liver transplantation. The fact that HPS is associated with worse survival in patients with liver disease, particularly in those patients who do not receive liver transplantation, underscores the need to develop effective medical therapies. These findings may further justify screening for HPS using arterial blood gases and contrast-enhanced transthoracic echocardiography in all candidates for liver transplantation [51] and argue for consideration of carefully phenotyped HPS (irrespective of the P_{aO2}) in the prioritisation for liver transplantation.

There are several limitations to this study. First, we only included patients being considered for liver transplantation in the USA, which is a select population. Differences in the makeup of the liver transplantation populations and allocation policies in other countries warrant similar studies of HPS in those populations. Second, we excluded some patients from the main analyses in order to create “clean” phenotypes. However, survival analyses including all patients still suggested an increased risk of death for patients with HPS. Third, it is possible that unmeasured or imprecisely measured variables could have confounded the findings, quantified by the E-values. Hypothesis-generating analyses suggested that angiopoietin 2, vWF and other angiogenic biomarkers could explain how HPS reduces survival. We did not correct for multiple comparisons owing to the multiple hypotheses investigated, therefore type 1 error is possible. Finally, although this was a multicentre study and conducted over several years, the sample size was relatively small. Still, this cohort remains one of the largest (and only) prospective multicentre studies of HPS with extensive protocolised lung and heart phenotyping to our knowledge.

In summary, HPS is common in patients being evaluated for liver transplantation and is associated with worse functional status, exercise capacity and overall survival. Understanding the mechanisms of the adverse effects of HPS and developing effective medical therapies merit high priority to improve the outcomes of patients with advanced liver disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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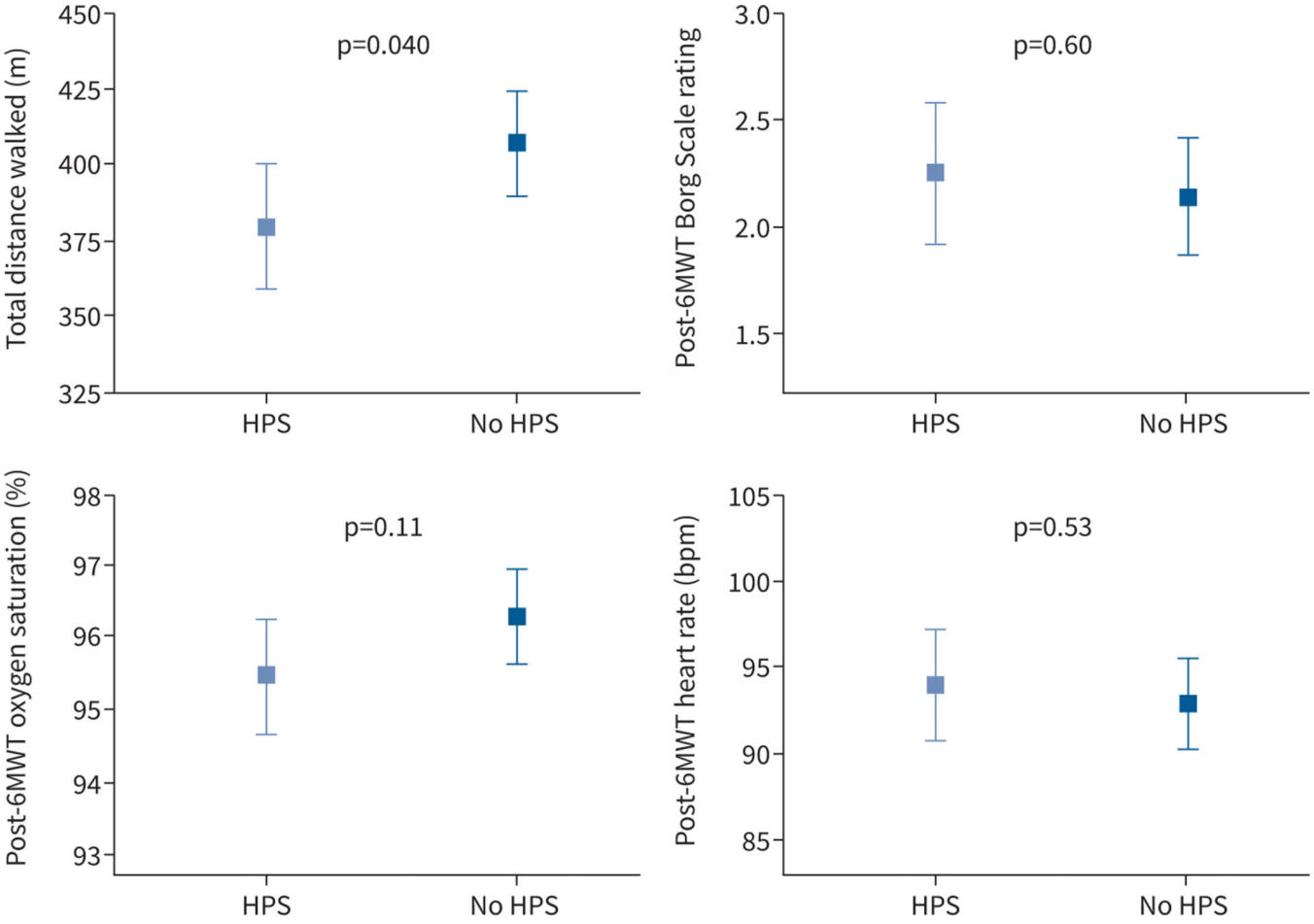

FIGURE 1.

Least square means and 95% confidence intervals of 6-min walk test (6MWT) parameters. All values are adjusted for age, sex, Model for End-Stage Liver Disease (MELD)-Na and baseline values (other than distance). HPS: hepatopulmonary syndrome.

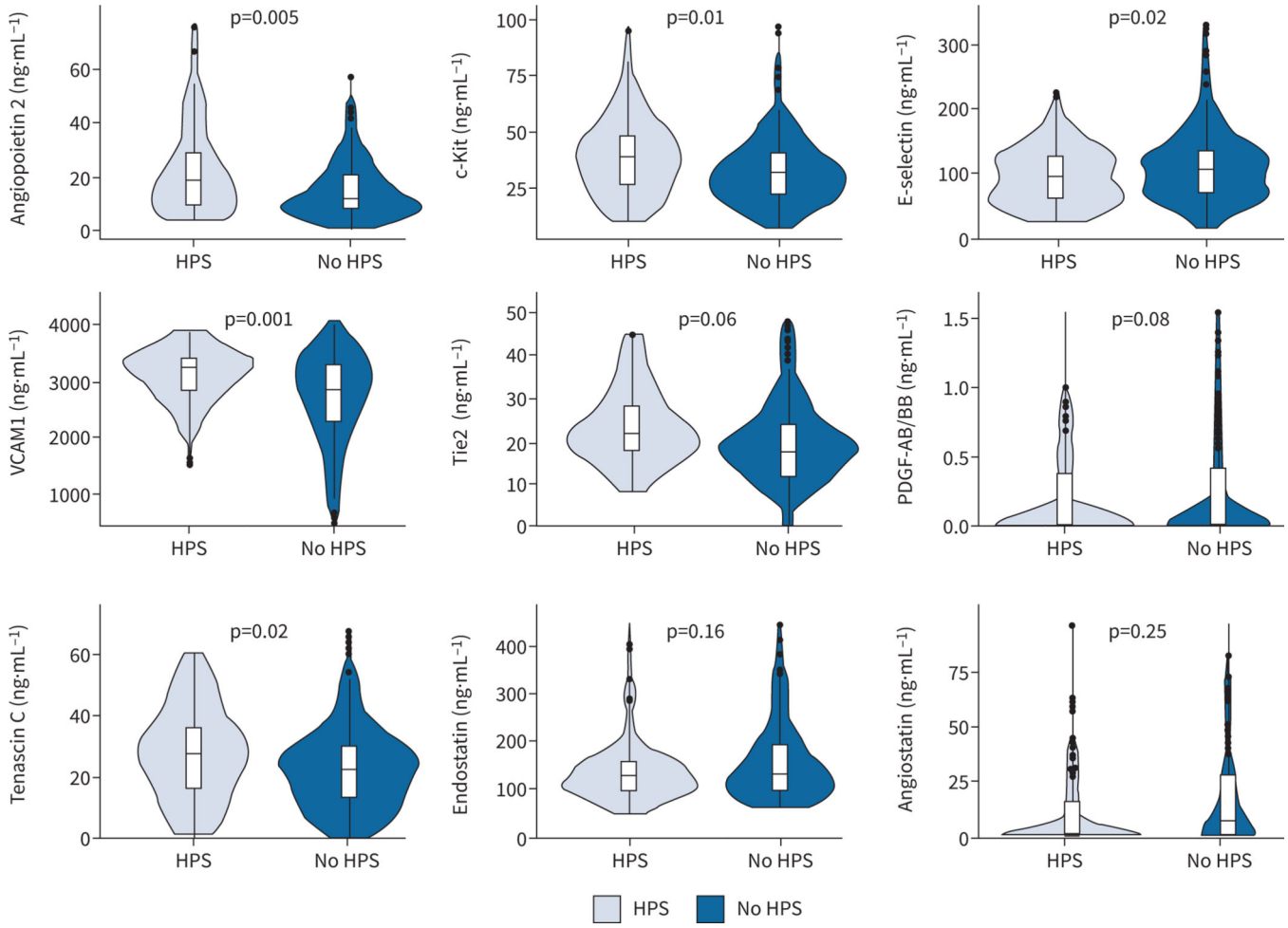

FIGURE 2.

Violin plots of selected angiogenesis biomarkers. Boxes are interquartile ranges with median. Whiskers are observations within 1.5×interquartile range. Plots of platelet-derived growth factor (PDGF)-AB/BB, endostatin and angiostatin only show data that are within 1.5×interquartile range. p-values are from multivariable linear regression models adjusted for age and Model for End-Stage Liver Disease-Na score. HPS: hepatopulmonary syndrome; C-Kit: tyrosine protein kinase Kit; VCAM1: vascular cell adhesion molecule 1; Tie2: TEK tyrosine kinase, endothelial.

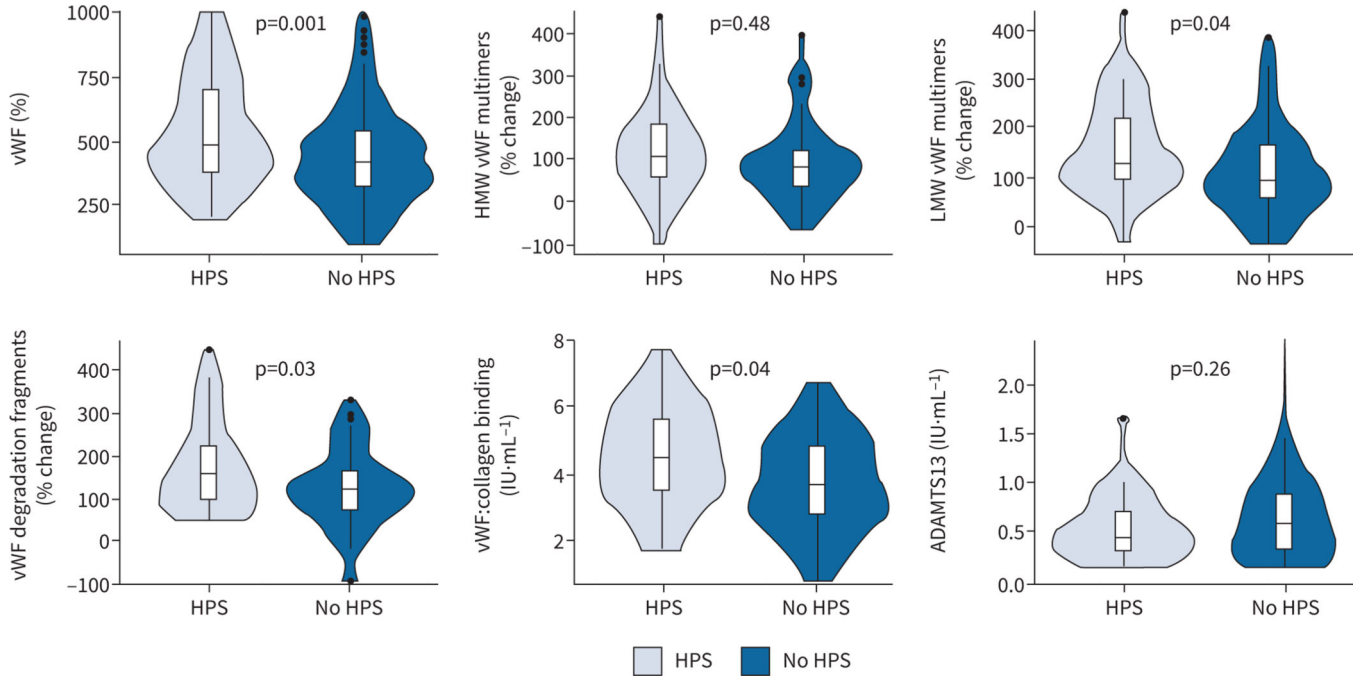

FIGURE 3.

Violin plots of von Willebrand factor (vWF) biomarkers. Boxes are interquartile ranges with median. Whiskers are observations within 1.5×interquartile range. p-values are from multivariable linear regression models adjusted for age and Model for End-Stage Liver Disease-Na score. HPS: hepatopulmonary syndrome; HMW: high molecular weight; LMW: low molecular weight; ADAMTS13: ADAM metallopeptidase with thrombospondin type 1 motif 13.

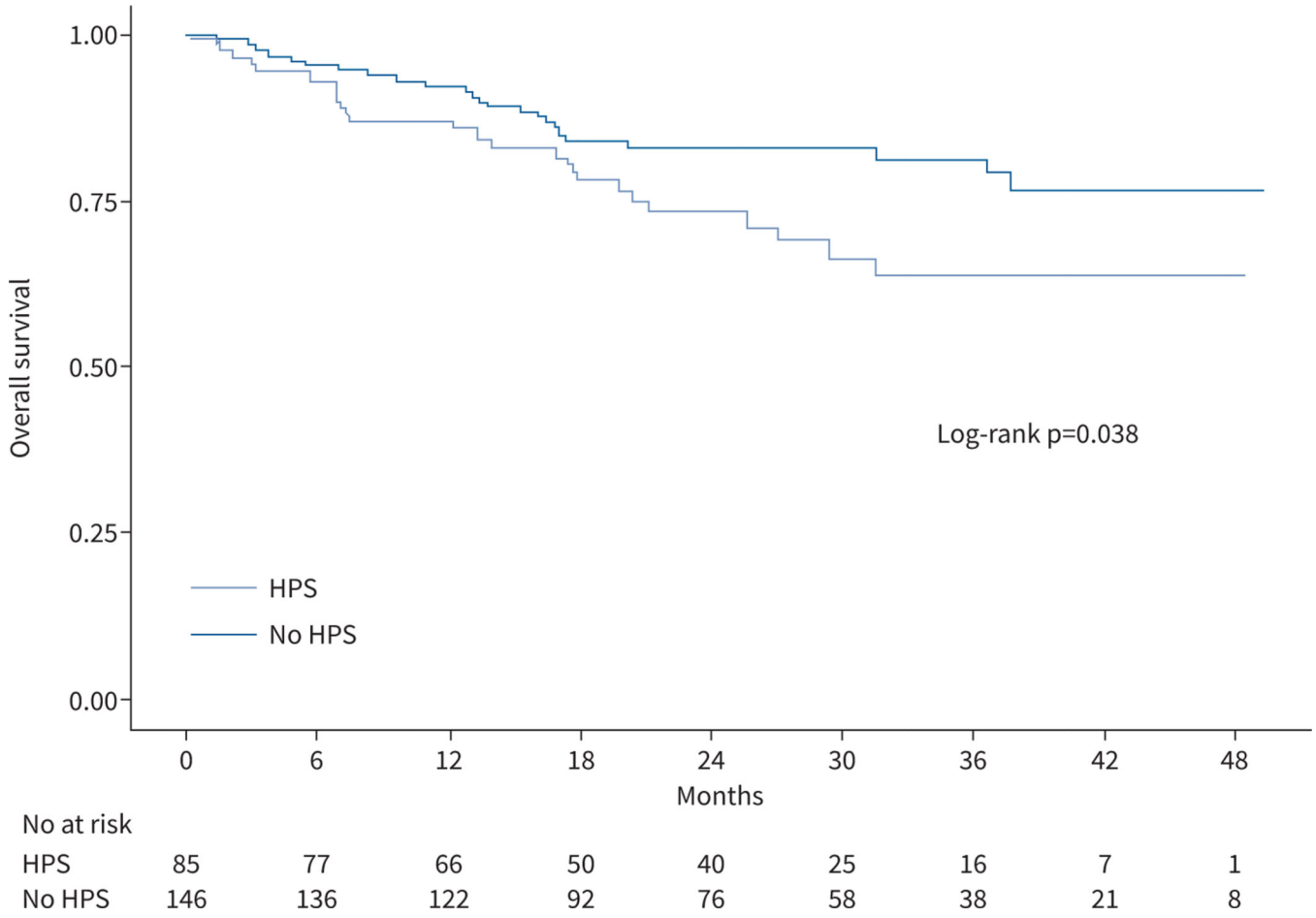

FIGURE 4.
Kaplan–Meier curves comparing patients with hepatopulmonary syndrome (HPS) and liver disease controls.

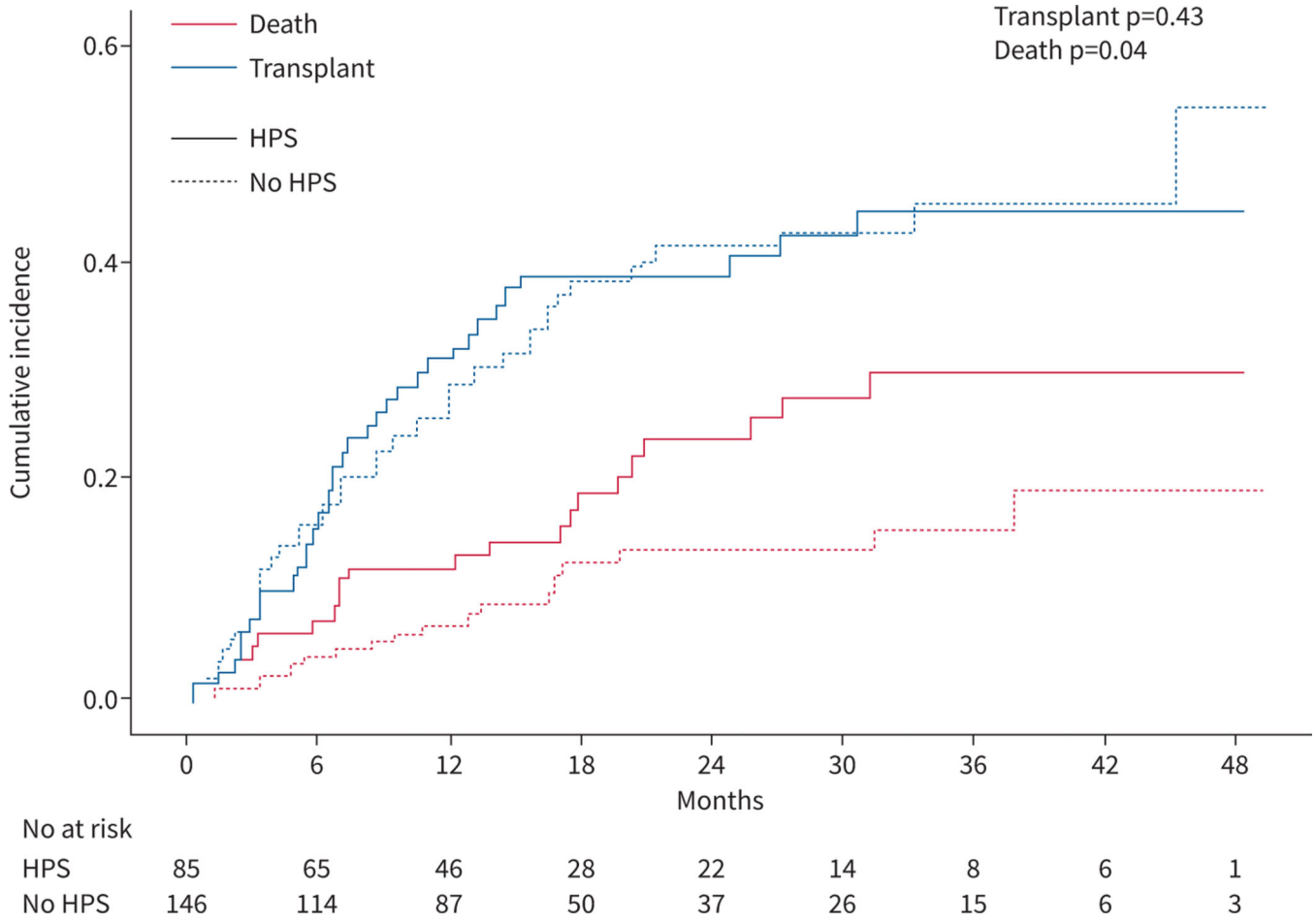

FIGURE 5.
Predicted cumulative incidences of death and liver transplantation for patients with hepatopulmonary syndrome (HPS) and liver disease controls.

TABLE 1

Demographics, liver disease characteristics and past medical history

Variable	Total participants, n	HPS (n=85)	No HPS (n=146)	p-value[#]
Age (years), mean±SD	231	55.2±9.4	57.6±8.9	0.06
Female	231	33 (38.8)	40 (27.4)	0.07
Race/ethnicity	231			0.02
Non-Hispanic white		70 (82.4)	95 (65.1)	
Hispanic white		12 (14.1)	29 (19.9)	
Non-Hispanic black		2 (2.4)	16 (11.0)	
Other		1 (1.2)	6 (4.1)	
Born in the USA/Puerto Rico	230	74 (88.1)	135 (92.5)	0.27
Language spoken in the household	230			1.0
English		78 (92.9)	133 (91.1)	
Spanish		1 (1.2)	3 (2.1)	
Other		5 (6.0)	10 (6.8)	
Education	230			0.51
No schooling or Grades 1–11		11 (13.1)	24 (16.4)	
High school or GED degree		26 (31.0)	42 (28.8)	
Some college education or technical/vocational certificate		18 (21.4)	25 (17.1)	
Associate or Bachelor’s degree		26 (31.0)	42 (28.8)	
Professional or Graduate degree		3 (3.6)	13 (8.9)	
Family income for past 12 months (USD)	230			0.33
⩽19999		18 (21.4)	39 (26.7)	
20000–49999		22 (26.2)	31 (21.2)	
50000–99999		17 (20.2)	28 (19.2)	
⩾100000		14 (16.7)	35 (24.0)	
Unknown		13 (15.5)	13 (8.9)	
Aetiology of liver disease				
Alcohol	231	34 (40.0)	48 (32.9)	0.28
Hepatitis C infection	231	37 (43.5)	62 (42.5)	0.88
Autoimmune hepatitis	231	4 (4.7)	6 (4.1)	1.0
Non-alcoholic fatty liver disease	231	20 (23.5)	33 (22.6)	0.87
Hepatitis B infection	231	1 (1.2)	6 (4.1)	0.43
Primary sclerosing cholangitis	231	4 (4.7)	8 (5.5)	1.0
Primary biliary cholangitis	231	9 (10.6)	6 (4.1)	0.05
Cryptogenic cirrhosis	231	3 (3.5)	11 (7.5)	0.22
Other	231	6 (7.1)	5 (3.4)	0.22
MELD-Na score, median (IQR)	227	15.0 (12.0–19.0)	14.0 (10.0–17.0)	0.006
History of liver disease complications				
Ascites	231	64 (75.3)	91 (62.3)	0.04
Varices	231	64 (75.3)	93 (63.7)	0.07
Variceal bleeding	231	29 (34.1)	42 (28.8)	0.40

Variable	Total participants, n	HPS (n=85)	No HPS (n=146)	p-value[#]
Encephalopathy	231	55 (64.7)	73 (50.0)	0.03
Multiple paracenteses	231	31 (36.5)	42 (28.8)	0.23
Spontaneous bacterial peritonitis	231	4 (4.7)	8 (5.5)	1.0
Hepatocellular carcinoma	231	22 (25.9)	58 (39.7)	0.03
Hepatic hydrothorax	231	10 (11.8)	13 (8.9)	0.48
Transjugular intrahepatic porto-systemic shunt	231	11 (12.9)	8 (5.5)	0.05
Past medical history				
Chronic obstructive pulmonary disease	231	5 (5.9)	7 (4.8)	0.76
Chronic bronchitis	231	7 (8.2)	7 (4.8)	0.30
Asthma	231	12 (14.1)	7 (4.8)	0.01
Venous thromboembolism	231	5 (5.9)	5 (3.4)	0.50
Diabetes mellitus	231	22 (25.9)	63 (43.2)	0.009
Hypertension	231	28 (32.9)	82 (56.2)	0.001
Hypercholesterolaemia	231	16 (18.8)	28 (19.2)	0.95
Congestive heart failure	231	3 (3.5)	8 (5.5)	0.75
Smoked at least 100 cigarettes in lifetime	230	53 (63.1)	82 (56.2)	0.30
Pack-years for ever-smokers, median (IQR)	106	22 (6–35)	11 (4–27)	0.12
Smoked in the last 30 days	231	13 (15.3)	16 (11.0)	0.34
Consumed alcohol	230	76 (90.5)	138 (94.5)	0.25
Duration of alcohol consumption (years), median (IQR)	214	30 (20–37)	32 (22–40)	0.12
Current alcohol use	231	5 (5.9)	12 (8.2)	0.51
Medications				
β-blockers	230	44 (51.8)	72 (49.7)	0.76
Spontaneous bacterial peritonitis prophylaxis/antibiotics	230	42 (49.4)	61 (42.1)	0.28
Bile acid resins	230	14 (16.5)	13 (9.0)	0.09
Midodrine	230	0 (0.0)	2 (1.4)	0.53

Data are presented as n (%), unless otherwise indicated. HPS: hepatopulmonary syndrome; GED: General Educational Development; MELD: Model for End-Stage Liver Disease; IQR: interquartile range.

[#]: Pearson's chi-squared test, Fisher's exact test, two-sample t-test, Wilcoxon rank sum test, as appropriate.

TABLE 2

Symptoms, signs, physical findings and laboratory evaluation

Variable	Total participants, n	HPS (n=85)	No HPS (n=146)	p-value[#]
Symptoms				
Dyspnoea	231	34 (40.0)	33 (22.6)	0.005
Chest pain	231	8 (9.4)	7 (4.8)	0.17
Orthopnoea	230	1 (1.2)	4 (2.7)	0.65
Palpitations	231	5 (5.9)	8 (5.5)	1.0
Syncope	231	2 (2.4)	2 (1.4)	0.63
Platypnoea	230	2 (2.4)	2 (1.4)	0.62
WHO functional class	231			<0.001
I		16 (18.8)	64 (43.8)	
II		47 (55.3)	57 (39.0)	
III		22 (25.9)	25 (17.1)	
IV		0 (0.0)	0 (0.0)	
Signs				
Cyanosis	231	7 (8.2)	1 (0.7)	0.004
Jaundice	231	43 (50.6)	34 (23.3)	<0.001
Lower extremity oedema	231	47 (55.3)	65 (44.5)	0.11
Clubbing	231	11 (12.9)	6 (4.1)	0.01
Spider angiomata	231	3 (3.5)	6 (4.1)	1.0
Asterixis	230	36 (42.4)	43 (29.7)	0.05
Ascites	231			0.47
Absent		45 (52.9)	89 (61.0)	
Mild-moderate		32 (37.6)	47 (32.2)	
Severe		8 (9.4)	10 (6.8)	
Encephalopathy	231			0.27
Absent		67 (78.8)	127 (87.0)	
Mild (I–II)		17 (20.0)	18 (12.3)	
Severe (III–VI)		1 (1.2)	1 (0.7)	
Physical examination, mean±SD				
Body mass index ($kg \cdot m^{-2}$)	231	31±7	30±7	0.22
Waist–hip ratio	218	1.0±0.1	1.0±0.1	0.67
Pulse (beats per min)	231	74±14	72±13	0.26
Respiratory rate (breaths per min)	230	15±3	16±3	0.17
Systolic blood pressure (mmHg)	231	121±16	124±18	0.26
Diastolic blood pressure (mmHg)	231	66±9	70±11	0.006
Oxygen saturation (%)	231	96±4	98±2	<0.001
Orthodeoxia [¶]	227	10 (12)	7 (5)	0.04
Laboratory results, median (IQR)				
Blood urea nitrogen ($mg \cdot dL^{-1}$)	209	14 (10–20)	15 (11–21)	0.19

Variable	Total participants, n	HPS (n=85)	No HPS (n=146)	p-value[#]
Creatinine (mg·dL^{-1})	230	0.9 (0.8–1.1)	1.0 (0.8–1.2)	0.06
Haemoglobin (g·dL^{-1})	231	11.8 (10.4–13.7)	12.4 (10.9–13.6)	0.52
Platelet count (10^9 per L)	229	86 (62–109)	92 (66–136)	0.10
International normalised ratio	228	1.4 (1.2–1.6)	1.3 (1.1–1.5)	0.002
Alanine aminotransferase (U·L^{-1})	230	38 (28–64)	46 (27–72)	0.33
Aspartate aminotransferase (U·L^{-1})	230	64 (41–98)	58 (36–92)	0.17
Total bilirubin (mg·dL^{-1})	230	2.4 (1.5–3.7)	1.5 (0.8–2.8)	<0.001
Direct bilirubin (mg·dL^{-1})	226	0.9 (0.6–1.6)	0.6 (0.2–1.1)	<0.001
Alkaline phosphatase (U·L^{-1})	230	149 (93–220)	148 (112–194)	0.98
Total protein (g·dL^{-1})	230	6.8 (6.4–7.3)	7.2 (6.6–7.6)	0.004
Albumin (g·dL^{-1})	230	3.0 (2.6–3.4)	3.2 (2.8–3.7)	0.01
Pulmonary function testing, mean±SD				
FVC (% pred)	231	88±10	91±12	0.15
FEV_1 (% pred)	231	89±11	89±12	0.68
FEV_1/FVC	231	0.77±0.05	0.78±0.06	0.26
Arterial blood gas, mean±SD				
pH	231	7.45±0.04	7.44±0.04	0.17
P_{aCO_2} (mmHg)	231	33±5	35±5	0.001
P_{aO_2} (mmHg)	231	78±13	92±14	<0.001
Alveolar–arterial oxygen gradient (mmHg), median (IQR)	231	26 (20–37)	12 (7–19)	<0.001

Data are presented as n (%), unless otherwise indicated. HPS: hepatopulmonary syndrome; WHO: World Health Organization; IQR: interquartile range; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; P_{aCO_2}: arterial carbon dioxide tension; P_{aO_2}: arterial oxygen tension.

[#]: Pearson's chi-squared test, Fisher's exact test, two-sample t-test, Wilcoxon rank sum test, as appropriate;

[¶]: increase of ⩾3% in oxygen saturation from pulse oximetry from sitting to supine position.

TABLE 3

Models for the risk of death

Models	HR (95% CI)	p-value	aHR (95% CI)	p-value[#]	E-value (for the limit of the CI)
Overall survival	1.80 (1.03–3.16)	0.04	1.79 (1.02–3.15)	0.04	2.35 (1.13)
Overall survival with transplant as a time-varying covariate	1.78 (1.02–3.13)	0.04	1.71 (0.97–3.00)	0.06	2.25 (1.00)
Transplant-free survival	1.99 (1.07–3.71)	0.03	1.84 (0.99–3.44)	0.05	2.42 (1.00)
Survival with transplant as competing risk (Fine–Gray model) [¶]	1.91 (1.06–3.45)	0.03	1.87 (1.04–3.35)	0.04	2.45 (1.20)
Multistate model [+]					
Transition from evaluation to liver transplant	1.11 (0.73–1.69)	0.62	1.03 (0.67–1.58)	0.89	
Transition from evaluation to death without liver transplant	1.99 (1.07–3.71)	0.03	1.84 (0.99–3.44)	0.05	2.42 (1.00)
Transition from liver transplant to death	0.94 (0.23–3.80)	0.93	0.99 (0.24–4.15)	0.99	

HR: hazard ratio; aHR: adjusted hazard ratio.

[#]: adjusted for age and Model for End-Stage Liver Disease-Na score;

[¶]: sub-distributional HR for mortality;

[+]: schema for multistate model is shown in supplementary figure S3.