Research Article

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von Willebrand factor antigen for detection of hepatopulmonary syndrome in patients with cirrhosis

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Background & Aims: Hepatopulmonary syndrome (HPS) occurs in 20–30% of patients with liver cirrhosis and is associated with a >2 fold increased mortality. Endothelial dysfunction seems to play a central role in its pathogenesis. von Willebrand factor antigen (vWF-Ag), an established marker of endothelial dysfunction, is significantly elevated in patients with liver cirrhosis, portal hypertension, and in experimental HPS. Aim of the present study was to evaluate the impact of vWF-Ag as a screening marker for presence of HPS in patients with stable cirrhosis.

Methods: 145 patients with stable liver cirrhosis were screened for presence of HPS in this prospective cohort type cross sectional diagnostic study. vWF-Ag and SaO_2 levels were assessed at time of screening for HPS. Criteria of HPS were fulfilled in 31 (21%) patients.

Results: vWF-Ag levels were significantly higher in patients with HPS compared to patients without HPS (p <0.001). Furthermore, vWF-Ag correlated significantly with gas exchange in HPS positive patients (p <0.05). vWF-Ag is an independent predictor of HPS after correction for sex, age, model for endstage-liver disease (MELD), and hepatic venous pressure gradient (HVPG) (OR per 1% increase of vWF-Ag: 1.02, 95% CI: 1.00–1.04, p <0.05). The best cut-off was 328% at a sensitivity of 100% and specificity of 53.5%; positive predictive value: 36.9%; negative predictive value: 100%.

Conclusions: HPS is associated with elevated vWF-Ag levels. vWF-Ag may be a useful screening tool for early detection of HPS. Further studies investigating vWF-Ag in HPS will be needed to confirm our findings.

Keywords: Hepatopulmonary syndrome; HPS; Pulmonary vascular disease; von Willebrand factor; vWF-Ag.

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Abbreviations: HPS, hepatopulmonary syndrome; vWF-Ag, von Willebrand factor antigen; MELD, model for endstage-liver disease; IPVD, intrapulmonary vasodilation; vWF, von Willebrand factor; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; $AaPO_2$, alveolar-arterial oxygen gradient; PaO_2, arterial oxygen tension; CEE, contrast-enhanced echocardiography; SaO_2, pulse oxygen saturation; IQR, interquartile range; AUROC, area under the ROC curve.

Introduction

Hepatopulmonary syndrome (HPS) is defined as impaired arterial oxygenation due to intrapulmonary vasodilation (IPVD) in patients with underlying liver disease [1–3]. These vascular alterations lead to ventilation-perfusion mismatch, limitation of oxygen diffusion, and right-to-left shunt. HPS is most frequently described in patients with liver cirrhosis. Prevalence of HPS in patients with cirrhosis ranges between 15 and 30 percent and mortality is more than 2 fold increased in comparison to patients with cirrhosis without HPS [1,2,4–6]. Currently, liver transplantation is the only curative therapeutic option [7].

The exact pathogenesis of HPS is not fully understood. Increased levels of endothelial nitric oxide synthase and enhanced expression of pulmonary endothelin-B receptors, both leading to elevated levels of the vasoactive nitric oxide in the pulmonary vascular bed and increased expression of heme oxygenase-1 resulting in enhanced production of the vasodilator carbon monoxide contribute to vascular alterations in HPS [3,8–13].

von Willebrand factor (vWF) is a plasma glycoprotein that plays a crucial role in haemostasis and is expressed by endothelium cells. Weibel-Palade bodies, the storage granules of endothelial cells, release both vWF and P-selectin (a cell adhesion molecule). vWF represents their activation and therefore is used as a marker for endothelial perturbation and dysfunction [14–18]. Endothelial cell activation is a broad term making reference to a number of stimuli including, e.g., vascular shear stress, infection, and hypoxia so that the endothelium undergoes changes, which imply an imbalance of vasoactive mediators as well as a proinflammatory and procoagulatory state [14–20]. Levels of von Willebrand factor antigen (vWF-Ag) are significantly elevated in patients with liver cirrhosis and portal hypertension and correlate with liver dysfunction and hepatic venous pressure gradient (HVPG) [15,21–23]. Increased number of lung microvessels and significantly elevated vWF levels were observed in a rat model of HPS [11]. Additionally, single nucleotide polymorphisms in the vWF-gene are significantly associated with HPS in patients with cirrhosis [24].

The clinical impact of vWF-Ag levels in patients with HPS has not been assessed so far. We hypothesized that presence of HPS is associated with increased peripheral blood levels of vWF-Ag. Aim of the present study was to evaluate the applicability of vWF-Ag

as a screening tool for the presence of HPS in patients with cirrhosis.

Patients and methods

Patients

Patients with liver cirrhosis were consecutively screened for presence of HPS in this cohort type cross sectional diagnostic study. Screening was performed at the outpatient clinic and normal ward of the Medical University of Vienna, Division of Gastroenterology and Hepatology, Department of Internal Medicine III between 2008 and 2011. The study was approved by the ethics committee of the medical university of Vienna.

Diagnosis of liver cirrhosis was made by histological or by combination of clinical signs (ascites, caput medusa, spider naevi, and others) and laboratory findings or by typical radiologic signs (typical morphological alterations of the liver or signs of portal hypertension in abdominal ultrasonography or computed tomography scan). Inclusion criteria were presence of liver cirrhosis and age over 18 years. Exclusion criteria were active infection or gastrointestinal bleeding within the last 2 weeks, presence of cardiovascular disease, pulmonary hypertension, hepatorenal syndrome, and implantation of a transjugular intrahepatic portosystemic shunt. Furthermore, we excluded patients with current treatment with beta-blockers, interferon, anticoagulants, and anti-platelet drugs, as well as patients with a significant obstructive ventilatory defect, defined as forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) <70% with FEV_1 percent predicted <80%, or a significant restrictive ventilatory defect, defined as FVC <70% of predicted, presence of intracardiac shunting or inadequate image quality in echocardiography.

Screening for HPS

All patients were screened for presence of HPS. HPS was defined by (1) positive contrast enhanced echocardiography (detection of IPVD), (2) presence of liver cirrhosis, and (3) alveolar-arterial oxygen gradient ($AaPO_2$) $\geqslant$15 mmHg ($AaPO_2$ $\geqslant$20 mmHg in patients aged >64 years) and arterial oxygen tension (PaO_2) <80 mmHg [1,25].

IPVD was assessed via transthoracic contrast-enhanced echocardiography (CEE) [1,26]. Agitated saline causes microbubbles of >10 μm in diameter that usually do not pass through the pulmonary capillary bed. Appearance of microbubbles, after injecting in a peripheral vein, first in the right heart, and within three to six heart actions in the left heart demonstrates abnormal vasodilation of the intrapulmonary capillary bed. Early (<3 heart actions) appearance of microbubbles in the left heart was considered as intracardiac shunting. These patients were excluded from the study as presence or absence of intrapulmonary shunting could not be judged by transthoracic CEE.

Arterial blood gas analysis (on room air) was performed at rest by arterial puncture in a standardized sitting upright position.

Portal pressure was assessed by measurement of HVPG (hepatic venous pressure gradient: difference between wedged hepatic venous pressure and free hepatic venous pressure) [22]. Clinically significant portal hypertension was defined as HVPG $\geqslant$12 mmHg [25].

Assessment of vWF-Ag levels and digital pulse oxygen saturation

vWF-Ag was determined by a fully automated STA analyser and vWF-Liatest (Diagnostic Stago, Paris, France) from peripheral venous blood samples (Reference value in adults: 60–180%) as described elsewhere [22,27].

Peripheral pulse oxygen saturation (SaO_2) was measured in all patients at rest, in a sitting upright position by using a standard pulse oxymeter (Draeger Infinity, Lübeck, Germany) applied to the index finger.

Statistical analysis

Continuous variables were described as median and 25–75% interquartile range (IQR), for categorical variables absolute and relative parameters are presented. Correlation analysis was performed using Spearman's correlation. Continuous variables were compared using Mann-Whitney U test and categorical variables were compared using χ^2 tests. We used multivariable logistic regression to assess predictors of HPS. The dependent variable was HPS (yes *vs.* no as defined above). We entered vWF as the main covariable (vWF-Ag in% as linear effect) and age (years), sex (female = 1, male = 0), MELD (as score ranging from 6 to 40), and HVPG (mmHg) as other covariates to the model. Hosmer-Lemeshow test for assessing goodness of fit in logistic regression was performed. The overall diagnostic test accuracy of vWF-Ag and SaO_2 was assessed by receiver operating characteristics expressed as their area under the curve (AUROC). We compared AUROCs of vWF-Ag and SaO_2 at several definitions of the gold standard diagnosis using standard non-parametric methods. Estimates of diagnostic test accuracy (sensitivity, specificity, positive predictive value, negative predictive value, and likelihood-ratios) were calculated using standard methods. For the purpose of a screening test we set the cut-off value for vWF-Ag and SaO_2 at the maximum sensitivity aiming at the optimised specificity. For data management and analyses we used MS Excel 2008 for Mac, SPSS 17 for Mac (SPSS, Inc. Chicago, IL), and Stata 11 for Mac (Stata Corp., College Station, TX). All p values reported are two sided and p <0.05 was considered significant.

All patients received the same HPS screening procedure and measurement of vWF-Ag and SaO_2 at the same time point. Experienced investigators performing HPS screening were blinded to the results of CEE, vWF-Ag, and SaO_2 and *vice versa*.

Results

Baseline characteristics

One-hundred forty five patients with liver cirrhosis were enrolled prospectively in this study. Demographic and clinical patient characteristics are summarized in Table 1.

The most common cause of cirrhosis in our cohort was alcoholic liver disease (58%) followed by hepatitis C virus infection (26%), and others such as autoimmune hepatitis, hepatitis B virus infection, or Wilson's disease (17%). 40 patients (28%) were classified as Child-Turcotte-Pugh A, 65 (45%) as Child-Turcotte-Pugh B and 40 (28%) as Child-Turcotte-Pugh C. Median model for endstage-liver disease (MELD) score for the total cohort was 11 (IQR 8–15).

52 out of 61 patients with invasive assessment of HVPG had clinically significant portal hypertension. HVPG did not differ between HPS positive and HPS negative patients (median 18 mmHg (IQR, 16 mmHg–22 mmHg) *vs.* 17 mmHg (IQR, 13 mmHg–22 mmHg) p = 0.6).

Furthermore, vWF-Ag levels correlated significantly with HVPG (r = 0.43, p <0.001), as illustrated in Fig. 1.

vWF-Ag levels were significantly higher in patients which developed complications of cirrhosis (ascites, hepatic encephalopathy grade 3 or 4, gastrointestinal bleeding, liver transplantation, and/or death) in a time period of 1 year follow up (median 381% (IQR 300%–423%) *vs.* 288% (IQR 227%–412%) p <0.01).

Prevalence of HPS

31 patients (21%) fulfilled criteria for HPS. Sex, age, height, weight, and aetiology of cirrhosis did not differ significantly between patients with and without HPS. Patients with HPS had significantly more advanced stages of liver cirrhosis assessed via MELD and Child-Pugh classification (Table 1). Patients with HPS had significantly impaired gas exchange by means of $AaPO_2$ and PaO_2.

vWF-Ag levels and HPS

HPS positive patients had significantly higher levels of vWF-Ag compared to HPS negative patients (median 423% (IQR, 387%–519%) *vs.* 315% (IQR, 248%–417); p <0.001). There was no significant correlation of vWF-Ag levels and MELD-score within the cohort of HPS positive subjects (r = 0.27, p = 0.14). vWF-Ag