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Sigrid Bachmann-Brandt
Isabel Bittner
Peter Neuhaus
Ulrich Frei
Ralf Schindler

Plasma levels of endothelin-1 in patients with the hepatorenal syndrome after successful liver transplantation

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S. Bachmann-Brandt · P. Neuhaus
Department of General and Transplant Surgery, Universitätsklinikum Charité, Campus Virchow Klinikum, Humboldt University, Berlin, Germany

I. Bittner · U. Frei · R. Schindler (✉)
Department of Nephrology, Universitätsklinikum Charité, Campus Virchow Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany
e-mail: ralf.schindler@charite.de
Tel.: + 49-30-450-53232
Fax: + 49-30-450-53993

Abstract The hepatorenal syndrome (HRS) is characterized by renal vasoconstriction leading to deterioration of renal function in patients with liver disease. A possible role of endothelin-1 (ET-1) in the pathogenesis of HRS has been suggested, but a correlation between ET-1 plasma levels and the development of HRS as well as the recovery from HRS following OLT has not been shown yet. We performed longitudinal measurements of ET-1 plasma levels in four groups of patients, 5 patients with HRS before and after orthotopic liver transplantation (OLT), 10 patients without HRS undergoing OLT, 20 patients with chronic renal failure but without liver disease, and 12 healthy controls. Before OLT, plasma levels of ET-1 were higher in patients with HRS (19.5 ± 8.6 ng/l, $P < 0.001$; $n = 5$) compared to patients without HRS (4.9 ± 1.1 ng/l; $n = 10$), normals (1.2 ± 0.18 ng/l; $n = 12$), and patients with chronic renal failure (2.4 ± 0.4 ng/l; $n = 20$). Patients with HRS compared to patients without HRS had higher levels for creatinine (2.42 ± 0.6 vs. 0.89 ± 0.05 mg/dl, $P < 0.05$), creatinine clearance (107 ± 9 ml/min vs. 44.6 ± 5.5 ml/min, $P < 0.001$), and bilirubin (11.4 ± 3.8 vs. 3.7 ± 1 mg/dl, $P < 0.05$) before OLT. Within one week after OLT, there was a rapid decrease in ET-1 levels in patients with HRS while creatinine and bilirubin levels decreased slower. Regression analysis revealed a weak correlation between serum creatinine and ET-1 ($r = 0.192$, $P = 0.04$) and a significant correlation between serum bilirubin and ET-1 ($r = 0.395$, $P < 0.001$). The means of the ET-1 levels decreases rapidly with improvement of liver function after OLT. Levels of ET-1 correlate with excretory liver function assessed by bilirubin. The fall in ET-1 levels preceding improvement of renal function further strengthens the concept of ET-1 being a causative factor in HRS.

Key words Endothelin-1 · Hepatorenal syndrome · Liver transplantation · Renal failure

Abbreviations *HRS* Hepatorenal syndrome · *ET-1* Endothelin-1 · *OLT* Orthotopic liver transplantation

Introduction

The HRS is characterized by intense renal vasoconstriction leading to renal dysfunction in patients with advanced liver failure [5]. The prevalence of HRS in liver recipients before transplantation is approximately 10% [9]. The pure functional nature of HRS is supported by the observation that kidneys from brain-dead patients with HRS can be successfully transplanted and function well in patients with endstage renal disease [18]. More-

over, after successful OLT of patients with HRS, urine volume increases within 3 days after OLT, and renal function improves although the glomerular filtration rate remains lower than in patients without HRS [14].

The pathogenesis of HRS is not fully understood [5]. Portal hypertension is accompanied by splanchnic and peripheral vasodilatation. Fluid losses into the peritoneal cavity may lead to reduced effective arterial blood volume, triggering the renin angiotensin system, the sympathetic nervous system and the secretion of antidiuretic hormone with the consequence of water and salt retention by the kidneys. However, unlike in prerenal azotemia, in HRS, renal function cannot be fully restored by the administration of fluids. Because of the observation of peripheral vasodilatation in the presence of renal vasoconstriction, it was suggested that the Endothelins may play a pathogenetic role in HRS [21]. The Endothelin family consists of three isoforms produced as large precursors preproendothelin that are cleaved by Endothelin-Converting-Enzyme. The renal vasculature is uniquely sensitive to ET, especially to ET-1. Infusion of 2.5 ng/kg per min ET-1 into normal volunteers leads to dramatic decreases in renal function but has only modest effects on systemic blood pressure [27]. Moore and colleagues reported increased plasma levels of ET-1 in patients with chronic liver diseases compared to normals [21]. Patients with liver disease and HRS had twice as high plasma levels of ET-1 compared to patients without HRS, indicating that ET-1 has a pathogenetic role in HRS. However, an association does not prove causal relationship, and the transversal nature of this study has been debated [1] since it did not follow patients with HRS after improvement of liver function, for example by liver transplantation. The present study is the first one providing longitudinal measurements of ET-1 in individual patients. If ET-1 is a pathogenetic factor in HRS, plasma levels of ET-1 should decrease and renal function should improve after improvement of liver function. To test this hypothesis, we performed longitudinal measurements of ET-1 plasma levels in patients with HRS undergoing OLT.

Methods

Study subjects

We studied two groups of patients with liver disease awaiting OLT. All patients except for one had liver failure stage Child B–C. Group 1 ($n = 10$) consisted of patients with serum-creatinine values < 1.2 mg/dl before transplantation. The cause of liver failure was hepatitis C ($n = 4$), hepatitis B ($n = 1$), M. Wilson ($n = 1$), alcoholic cirrhosis ($n = 1$), autoimmune hepatitis ($n = 1$), primary sclerosing hepatitis ($n = 1$) and Klatskin tumor ($n = 1$). Two of these patients developed acute renal failure after OLT and were excluded from the analysis.

Group 2 consisted of 5 patients with serum-creatinine values > 1.2 mg/dl before transplantation. Serum-creatinine increased within the 4 weeks before OLT in 3 patients and was steadily elevated for at least 2 months before OLT in two patients. The cause of liver failure was alcoholic cirrhosis ($n = 2$), hepatitis B ($n = 1$), autoimmune hepatitis ($n = 1$), and unknown ($n = 1$). Renal ultrasound revealed normal kidney size in all patients. In Group 2, the central venous pressure at the start of transplantation was 12 ± 2.3 mm Hg.

Group 3 consisted of 20 patients from the nephrology ward with different degrees of chronic renal insufficiency but without liver disease.

Different immunosuppressive protocols were used during the course of the study, all of them included steroids and either cyclosporine A (CyA) or tacrolimus.

As controls, 12 healthy subjects from the medical staff were studied.

Sample collection

For measurement of ET-1, blood samples were drawn between 8 and 9 a.m. In groups 1 and 2, blood was drawn before OLT and at least at weekly intervals up to 4 weeks after OLT. In groups 3 and 4, blood was drawn once. 10 ml of blood was drawn into tubes containing EDTA (Sigma-Aldrich GmbH, Deisenhofen, Germany, final concentration 1 mg/ml), chilled on ice, and immediately centrifuged at 4 °C. Plasma was obtained and stored at -70 °C until extraction.

For determination of creatinine and bilirubin in groups 1 and 2, blood was drawn immediately before OLT and at least at weekly intervals up to 4 weeks after OLT. After 4 weeks, patients were followed in the outpatient clinic at 3 and 6 months after OLT. Serum creatinine and bilirubin were determined as routine parameters in our central laboratory. GFR was calculated by the formula of Cockroft and Gault: Cr-Clearance = (140-age) · kg BW/(mg/dl serum-creatinine · 72).

Measurement of ET-1

ET-1 was extracted from plasma by a modification of the method of Moore et al. [21]. C18 SepPak columns (Waters Corp., Milford, MA) were rinsed with 5 ml acetonitrile/0.1 % triflouric acid followed by 5 ml methanol/0.1 % triflouric acid and destilled water/0.1 % triflouric acid. After application of 3 ml of plasma, columns were rinsed with destilled water/0.1 % triflouric acid and 40 % methanol/0.1 % triflouric acid. Endothelin was eluted with 3 ml 70 % methanol/0.1 % trifloruic acid/0.01 % triton X (Sigma). The eluate was dried under vacuum and resuspended in 500 µl of RIA buffer. ET-1 was measured in duplicate by RIA (RIK 6901, Peninsula Laboratories Inc., Belmont, CA) with a sensitivity of 1 pg/tube. Because 100 µl of the 500 µl RIA buffer used to resuspend 3 ml of plasma sample was added per tube, the sensitivity was 0.6 pg/ml plasma. The cross-reactivity of the RIA as outlined by the manufacturer is 17 % for Big-ET-1, 7 % for ET-2, and 7 % for ET-3.

Statistical analysis

ET-1 levels between groups 1–4 were analyzed by ANOVA followed by Bonferroni/Dunn analysis (StatView, Abacus Concepts Inc.). Comparisons of creatinine, calculated creatinine clearance, bilirubin and ET-1 between group 1 and 2 were performed by unpaired t-test. In case of multiple measurements within weekly intervals after OLT, data were averaged per week. Levels for creati-

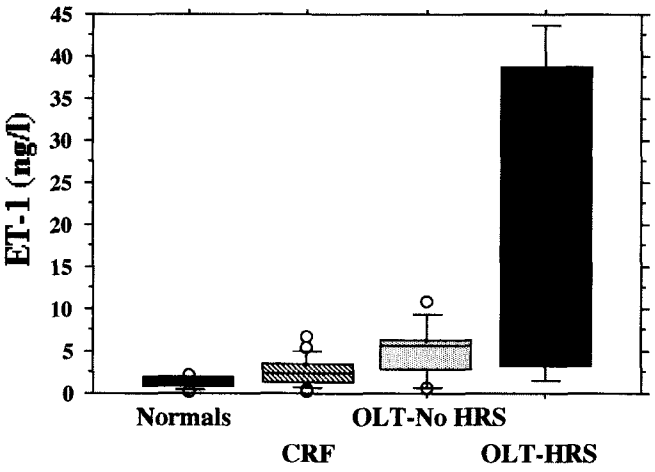

Fig. 1 Box plots of ET-1 levels in normal subjects ($n = 12$), patients with chronic renal failure (CRF, $n = 20$), patients before orthotopic liver transplantation without HRS ($n = 10$) and patients before orthotopic liver transplantation with HRS ($n = 5$). The plots denote the 10th, 25th, 50th, 75th and 90th percentile of the ET-1 levels. ET-1 levels in patients with HRS are significantly different from all other groups ($P < 0.001$)

nine, bilirubin and ET-1 after OLT were compared to levels before OLT by paired t-test in groups 1 and 2. Relations between ET-1, creatinine and bilirubin were analyzed by regression analysis using ANOVA tables (Statview). Unless otherwise indicated, data are given as Mean ± SEM.

Results

Plasma levels of ET-1 were significantly ($P < 0.001$) higher in patients with HRS (19.5 ± 8.6 ng/l) compared to normals (1.2 ± 0.18 ng/l), patients with chronic renal failure (2.4 ± 0.4 ng/l) and patients without HRS (4.9 ± 1.1 ng/l, Fig. 1). Although patients without HRS and patients with chronic renal failure had higher levels of ET-1 than normals, these differences were not statistically significant.

The mean serum creatinine in patients with chronic renal failure was 3.7 ± 0.75 mg/dl. Before OLT, patients with HRS compared to patients without HRS had higher levels for creatinine clearance (107 ± 9 ml/min vs. 44.6 ± 5.5 ml/min, $P < 0.001$), bilirubin (11.4 ± 3.8 vs. 3.7 ± 1 mg/dl, $P < 0.05$), and ET-1 (19.5 ± 8.6 vs. 4.9 ± 0.9 ng/l, $P < 0.05$; Fig. 2). The serum-creatinine before transplantation was 2.42 ± 0.6 mg/dl in group 1 and 0.89 ± 0.05 mg/dl in group 2 ($P < 0.05$).

In group 1, the mean urinary sodium excretion in 24-hour urine collections at the time of evaluation for transplantation was 102 ± 16 mmol/l. In group 2, the mean urinary sodium excretion in 24-h urine collections before transplantation was 42 ± 14.5 mmol/l ($P < 0.05$ vs. group 1) while on diuretic therapy with furosemide and spironolactone.

After OLT, there was a sharp decrease of ET-1 plasma levels within the first week for patients with HRS; however, because only 5 patients could be studied, these differences were not statistically significant. In patients without HRS, ET-1 levels were significantly lower at 3 weeks after OLT compared to pre-OLT (Fig. 3). Serum creatinine decreased significantly within the first week after OLT in patients with HRS but was not significantly different from pre-OLT values at later time points (Fig. 4). In patients without HRS, serum creatinine increased slightly after OLT, although not significantly (Fig. 4). Serum creatinine was significantly different between groups before and at 1 week after OLT (Fig. 4). At 3- and 6 months after OLT, serum creatinine was almost identical in the two groups. Bilirubin de-

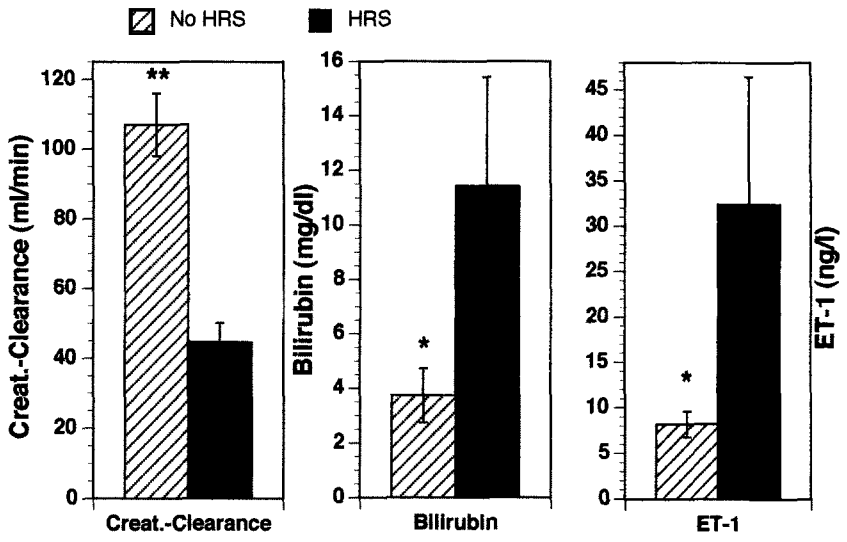

Fig. 2 Values for creatinine clearance, bilirubin and ET-1 for patients with and without HRS before liver transplantation. Means ± SEM, * indicates $P < 0.05$ and ** indicates $P < 0.01$ vs. patients with HRS

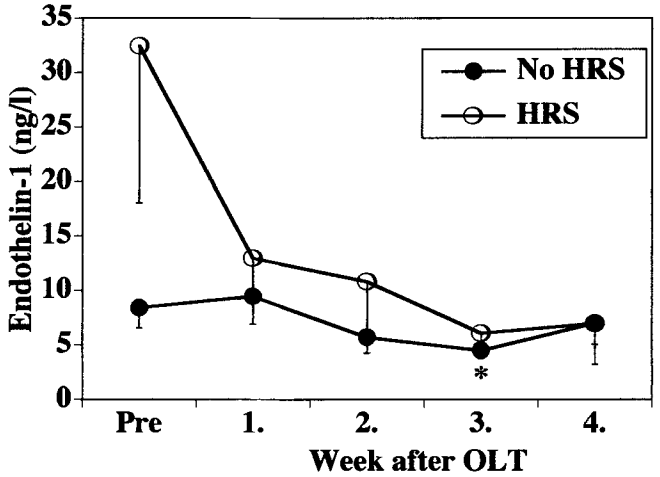

Fig. 3 ET-1 levels before- and up to 4 weeks after OLT. Means ± SEM, * indicates $P < 0.05$ vs. pre-OLT

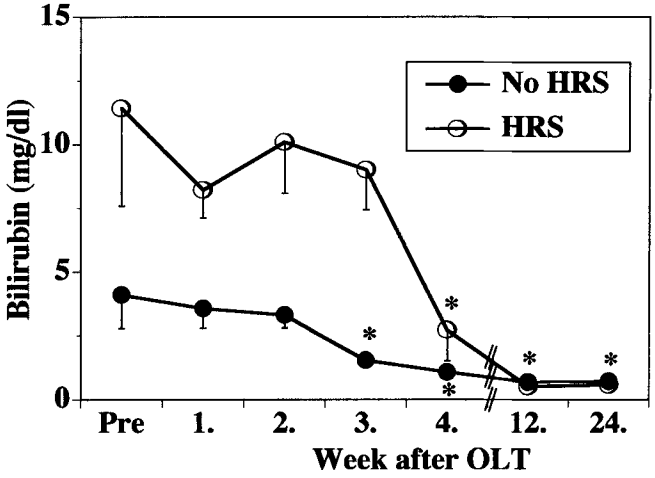

Fig. 5 Bilirubin levels before- and up to 6 months after OLT. Means ± SEM, * indicates $P < 0.05$ vs. pre-OLT; # indicates $P < 0.05$ between groups

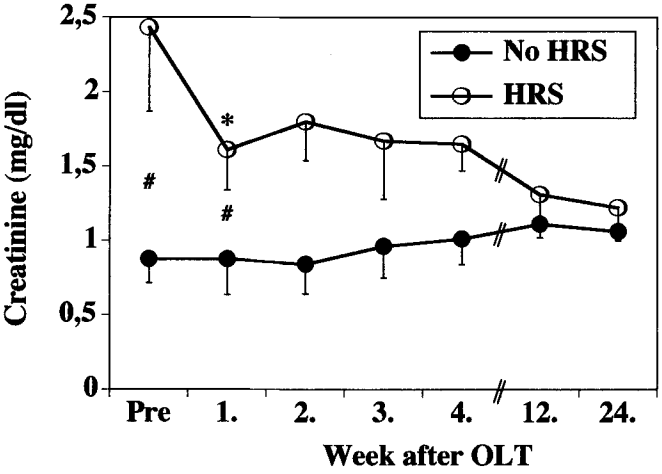

Fig. 4 Serum-creatinine before- and up to 6 months after OLT. Means ± SEM, * indicates $P < 0.05$ vs. pre-OLT; # indicates $P < 0.01$ between groups

creased in both groups that underwent transplantation after OLT, and was significantly lower from pre OLT values at 3 weeks and later, for patients without HRS, and at 4 weeks and later for patients with HRS (Fig. 5). Bilirubine levels were significantly different between groups before and at 1, 2 and 3 weeks after OLT (Fig. 5).

Regression analysis for all measurements ($n = 111$) in patients that underwent transplantation revealed a weak correlation between serum creatinine and ET-1 ($r = 0.192$, $P = 0.04$; Fig. 6). When patients with chronic renal failure but without liver disease were included in the analysis, there was no significant correlation between creatinine and ET-1 ($r = 0.09$). A significant correlation was observed in transplanted patients between serum bilirubin and ET-1 ($r = 0.395$, $P < 0.001$; Fig. 6).

Discussion

The present study demonstrates that 1. ET-1 levels are higher in patients with HRS than without HRS; 2. bilirubin levels are higher in patients with HRS than without HRS; 3. ET-1 levels correlate with bilirubin levels; and 4. ET-1 levels decrease rapidly after OLT, while renal and excretory liver function improve slower. The functional nature of renal impairment in patients with HRS is indicated by the almost identical creatinine values in both groups undergoing transplantation at 3 and 6 months after OLT. This is the first study reporting longitudinal measurements of ET-1 after liver transplantation in patients with HRS.

Somewhat surprising was the rapid decrease of ET-1 after OLT in patients with HRS although all patients were treated with either CyA or tacrolimus. Both of these agents have been reported to induce ET-1 in endothelial cells [3, 10, 22]. ET-1 plasma levels increase after oral administration of CyA or tacrolimus in recipients of solid organ transplants and paralleled CyA/tacrolimus-levels [11, 19]. In the present study, ET-1 levels were determined strictly in the morning before administration of CyA or tacrolimus to minimize any short term effects of these drugs on ET-1 levels. Because ET-1 levels declined rapidly after OLT despite of the administration of immunophilins, therapy with CyA or tacrolimus appears to have only minor effects on ET-1 plasma levels. In accordance, Textor et al. reported no increase in ET-1 plasma levels 12 months after OLT in patients without HRS compared to pre-transplant levels; only urinary ET-1 increased modestly both in CyA- and in tacrolimus treated patients [28].

Increased levels of ET-1 in patients with liver disease may be the result of increased production and/or de-

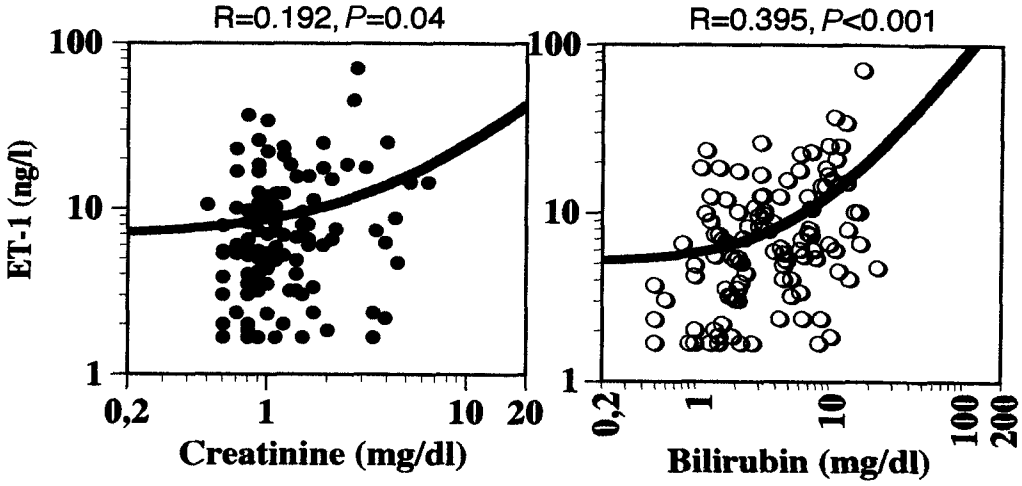

Fig. 6 Correlation between serum creatinine and ET-1 or bilirubin and ET-1 for all measurements (n = 111) in transplanted patients

creased elimination of ET-1. ET-1 is synthesized not only by endothelial cells as originally described, but also in other cell types such as macrophages and organs such as brain, lung, liver and kidneys [8]. Renal endothelial cells remain an important source for ET-1 because of their capacity to secrete ET-1 in response to stimuli such as acidosis [29]. Studies in humans demonstrate a positive AV-difference for ET-1 plasma levels (i.e., net production) during infusion of Big-ET-1 only for the kidney, but not for other organs [12]. Moore et al. reported higher levels of ET-1 in the renal vein than in the renal artery in patients with liver disease [21]. Thus, the synthesis of ET-1 in the vasoconstricted kidneys may be increased in liver failure. Another site of production of ET-1 is the splanchnic vasculature congested by portal hypertension [15, 24]. In the dog, Kawamura et al. reported an increase of ET-1 levels in the portal vein that correlated with the degree of portal hypertension [15] after clamping the portal vein. The rapid decrease of ET-1 in patients with HRS in the present study within one week after OLT (when renal function and bilirubin were still elevated) may be the effect of reversing portal congestion after OLT.

On the other hand, there are also indications that elimination of ET-1 may be diminished in liver disease. In rats, radioactive ET-1 injected into the left ventricle is taken up predominantly by the lungs, the liver and the kidneys [2]. Organ extracts from lungs and kidneys, but less from livers degrade ET-1 in vitro [25], indicating that the lung is the major clearance site for ET-1. Patients with chronic liver diseases have substantial pulmonary shunting of up to 20% of the cardiac output that is reversed after OLT [7]. Thus, the lungs as important eliminating organs for ET-1 may be bypassed in liver disease. The correlation between bilirubin and ET-1 observed in the present study indicates that levels of ET-1 are influenced by the excretory liver function. A similar correlation between bilirubin and ET-1 has been described by some [23] but not all authors [13]. Whether this association is direct (diminished clearance of ET-1 by the liver) or indirect (more pulmonary shunting with increasing severity of liver failure) cannot be determined by our data.

Our data do not support the view that elevated levels of ET-1 are simply the consequence of accumulation of ET-1 in renal failure, as suggested previously [4]. First, in accordance with previous reports [21], we observed only minor elevations of ET-1 levels in patients with chronic renal failure. Second, levels of ET-1 dropped rapidly after OLT when renal function was still impaired. Third, there was only a weak correlation between serum creatinine and ET-1 levels in patients that underwent transplantation. This is in contrast to other studies who did find a correlation between creatinine and ET-1 in patients with liver disease [20]. In this study [20], only single measurements were performed in 11 patients studied. Our results using 111 longitudinal measurements in 15 patients after OLT suggest only a very weak correlation between creatinine and ET-1. Thus, the contribution of renal failure to elevated levels of ET-1 appears to be small.

ET-1 may not be the only pressor agent responsible for renal vasoconstriction in HRS. Kitamura could not find a correlation between ET-1 plasma levels and renal vasoconstriction assessed by Duplex ultrasonography [16]. Rather, a putative not yet identified pressor substance, or a decrease in vasodilatory substances such as NO and prostaglandins, may contribute to the altered renal hemodynamics in HRS [6]. The present study suggests that ET-1 is an important pathogenetic factor in HRS but maybe not the only one. The role of Endothelins in HRS should be further addressed by the administration of ET- receptor antagonists to fulfil the second of Koch's postulates, i.e., blocking of a pathogen ameliorates the disease [17]. Preliminary results have been reported recently [26].

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