Decreased heart rate variability in patients with cirrhosis relates to the presence and degree of hepatic encephalopathy

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Mani AR, Montagnese S, Jackson CD, Jenkins CW, Head IM, Stephens RC, Moore KP, Morgan MY. Decreased heart rate variability in patients with cirrhosis relates to the presence and degree of hepatic encephalopathy. Am J Physiol Gastrointest Liver Physiol 296: G330–G338, 2009. First published November 20, 2008; doi:10.1152/ajpgi.90488.2008.—Heart rate variability (HRV) is decreased in several clinical settings associated with either systemic inflammation or neuropsychiatric impairment. The possibility that the changes in HRV observed in patients with neuropsychiatric impairment might relate to the overproduction of inflammatory cytokines does not seem to have been considered in the studies undertaken to date. HRV is decreased in patients with liver cirrhosis but its relationship to the impairment of neuropsychiatric performance, commonly observed in these patients, is unknown. The aim of this study was to investigate the relationship between HRV, hepatic encephalopathy, and production of inflammatory cytokines in patients with cirrhosis. Eighty patients with cirrhosis [53 men, 27 women; mean (±SD) age 54 ± 10 yr], classified as neuropsychiatrically unimpaired or as having minimal or overt hepatic encephalopathy, and 11 healthy subjects were studied. HRV was assessed by applying Poincaré plot analysis to the R-R interval series on a 5-min ECG. Inflammatory cytokines (TNFα, IL-6, IL-10, and IL-12) were measured in a subgroup of patients. Long-term R-R variability was significantly decreased in the patients with cirrhosis, in parallel with the degree of neuropsychiatric impairment (P < 0.01) and independently of the degree of hepatic dysfunction (P = 0.011). The relative risk of death increased by 7.7% for every 1-ms drop in this variable. Plasma levels of IL-6 significantly correlated with indexes of both HRV and neuropsychiatric performance. The changes observed in HRV and in neuropsychiatric status in patients with cirrhosis are significantly correlated, most likely reflecting a common pathogenic mechanism mediated by inflammatory cytokines.

inflammatory cytokines; electroencephalogram; linear/nonlinear dynamics; Psychometric tests; systemic inflammatory responses

HEART RATE VARIABILITY (HRV) is a measure of the physiological fluctuation of the cardiac cycle over time, which reflects the output of the complex control of the heart mediated by the autonomic nervous system (1, 21). Contrary to the predictions of the classical concept of homeostasis, the output of a wide variety of physiological systems, such as the normal heart beat, fluctuates in a complex manner, even under resting conditions (1, 21). Deceased variability or increased regularity of the cardiac rhythm has been reported in different clinical settings associated with systemic inflammation and increased production of inflammatory cytokines, such as sepsis, diabetes mellitus, ischemic heart disease, congestive heart failure, and hepatic failure; in these contexts, it is a negative predictor of outcome (5, 6, 22–24, 31).

The underlying mechanism of decreased HRV during systemic inflammation is unknown. However, it appears that the inflammatory cytokine IL-6 might play a role, since circulating levels of IL-6 correlate significantly with indexes of depressed HRV in various clinical conditions (5, 22, 54).

HRV is also decreased in patients with chronic liver disease (6, 12, 17, 25, 32, 37, 39). Plasma concentrations of inflammatory cytokines are increased in these patients, even in the absence of active infection (19, 50, 55), and this activation of inflammatory mediators might explain the decrease in HRV observed in these patients.

Decreased HRV has also been reported in several neuropsychiatric conditions, such as dementia (29, 62), mood disorders in postmenopausal women (28), and depression in patients with ischemic heart disease (58). Several mechanisms have been proposed to explain reduced heart rate fluctuations in these patients (28, 29, 58, 62). However, the possibility that the changes in HRV observed in patients with neuropsychiatric impairment might relate to the overproduction of inflammatory cytokines does not seem to have been considered in the studies undertaken to date. This is surprising given that increased production of inflammatory cytokines is known to occur in a number of neuropsychiatric disorders (4, 11, 20, 26, 57).

Hepatic encephalopathy is a neuropsychiatric syndrome that develops in patients with liver disease. It has recently been suggested that inflammatory mediators might play a crucial role in the pathogenesis of this syndrome (27, 42, 51). Serum levels of inflammatory cytokines have been shown to correlate with the severity of encephalopathy in patients with cirrhosis (43, 44). Both the presence of hepatic encephalopathy and the reduction in HRV have negative prognostic value in this patient population (7, 17). However, it is unknown whether any relationship exists between HRV parameters, neuropsychiatric impairment, and inflammatory cytokines in this clinical context.

The present study was undertaken to explore the relationship between HRV and neuropsychiatric performance in patients with cirrhosis in order to test the hypothesis that the decrease in HRV correlates with the presence and degree of hepatic encephalopathy.

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en cephalopathy via circulating inflammatory cytokines was explored in a subset of the same patient population.

METHODS

Study Population

The patient population comprised 80 individuals [53 men, 27 women; mean (±1 SD) age 54 ± 10 yr], with biopsy-proven cirrhosis referred for assessment of their neuropsychiatric status. The etiology of the liver disease was determined by use of clinical, laboratory, radiological and histological variables. The functional severity of the liver injury was assessed by using Pugh’s modification of the Child’s grading system (48). Patients were excluded from the study if they were under 16 or over 80 yr of age; had misused alcohol or suffered a significant upper gastrointestinal hemorrhage within the preceding 3 mo; had a history of diabetes mellitus, pancreatitis, anemia, cardiovascular/cerebrovascular disease, or systemic hypertension, significant head injury, or recent infection; or were taking neuroactive drugs or drugs known to affect the cardiac rhythm such as propranolol. The reference population comprised 11 healthy volunteers (5 men, 6 women) of mean age 49 ± 10 yr. None had a history, clinical, or laboratory evidence of alcohol misuse, chronic liver disease, or heart disease; none drank alcohol in excess of 20 g/day or took prescription medication.

Neuropsychiatric Assessment

Neuropsychiatric status was assessed in a single 90- to 120-min sitting. Patients’ mental state was evaluated by the West Haven criteria (15). Psychometric performance was assessed, under standardized conditions, by Number Connection Tests A and B (14), and the Digit Symbol subtest of the Wechsler Adult Intelligence Scale (59). Psychometric test results were scored in relation to age- and educational reference ranges. A significant upper gastrointestinal hemorrhage within the preceding 3 mo; had a history of diabetes mellitus, pancreatitis, anemia, cardiovascular/cerebrovascular disease, or systemic hypertension, significant head injury, or recent infection; or were taking neuroactive drugs or drugs known to affect the cardiac rhythm such as propranolol. The reference population comprised 11 healthy volunteers (5 men, 6 women) of mean age 49 ± 10 yr. None had a history, clinical, or laboratory evidence of alcohol misuse, chronic liver disease, or heart disease; none drank alcohol in excess of 20 g/day or took prescription medication.

Electroencephalograms (EEGs) were recorded, under standardized conditions, by using silver-silver chloride electrodes placed according

![Fig. 1. Poincaring plot is a graphical presentation of the correlation between the consecutive R-R intervals of the ECG [x-axis: R-R (n); y-axis: R-R (n+1)]. An ellipse is fitted on to the line of identity at 45° to the normal axis. The standard deviation of the points perpendicular to the line of identity, denoted by SD1, describes short-term R-R variability, which relates, largely, to the effects of respiration on vagal drive. The standard deviation along the line of identity, denoted by SD2, describes long-term R-R variability, which relates to all other heart rate changes. If the heart rhythm is regular then the points on the plot will be located close to the lines of identity.](image)

| Table 1. Demographic and assessment variables in the reference population and in the patients with cirrhosis, by degree of hepatic encephalopathy. |
|---|---|---|---|---|---|---|---|---|---|
| Gender, % men | Study Population | | Neuropsychiatric status | | | | | | |
| | Healthy volunteers | n (n) | 44 | | | | | | |
| | Overt HE | n (n) | 25 | | | | | | |
| | Patients with cirrhosis | n (n) | 54 | | | | | | |
| | Unimpaired | | 44 | | | | | | |
| | Minimal HE | n (n) | 13 | | | | | | |
| | Over HE | n (n) | 18 | | | | | | |
| | Age, yr | 49 (10-68) | 54 (26-87) | 57 (27-53) | 56 (40-66) | 53 (12-79) | 50 (25-67) | 52 (12-87) | 50 (26-110) |
| | Gender | 54 (53 men) | 54 (5 men) | 54 (5 men) | 54 (5 men) | 54 (5 men) | 54 (5 men) | 54 (5 men) | 54 (5 men) |
| | Pugh’s score, 5–15 | 44 (10.7) | 42 (12.4) | 43 (12.3) | 43 (11.9) | 40 (11.9) | 41 (12.2) | 40 (12.6) | 41 (10.8) |
| | NCT A, s | 11 (10.2) | 12 (10.2) | 12 (10.2) | 12 (10.2) | 10 (10.2) | 11 (10.2) | 10 (10.2) | 11 (10.2) |
| | NCT B, s | 11 (10.2) | 12 (10.2) | 12 (10.2) | 12 (10.2) | 10 (10.2) | 11 (10.2) | 10 (10.2) | 11 (10.2) |
| | Alpha, % | 46 (88) | 46 (88) | 46 (88) | 46 (88) | 46 (88) | 46 (88) | 46 (88) | 46 (88) |
| | Beta, % | 14 (13) | 14 (13) | 14 (13) | 14 (13) | 14 (13) | 14 (13) | 14 (13) | 14 (13) |
| | Delta, % | 5 (9) | 5 (9) | 5 (9) | 5 (9) | 5 (9) | 5 (9) | 5 (9) | 5 (9) |
| | Theta, % | 13 (24) | 13 (24) | 13 (24) | 13 (24) | 13 (24) | 13 (24) | 13 (24) | 13 (24) |
| | MDF, Hz | 43 (1.6) | 43 (1.6) | 43 (1.6) | 43 (1.6) | 43 (1.6) | 43 (1.6) | 43 (1.6) | 43 (1.6) |
| | Significance of the differences between series: a p < 0.05; b p < 0.01; bbb p < 0.001. Significance of the differences between patients with minimal and patients with overt HE: cc p < 0.01. |

Data are expressed as means ± SD (range). Data for Number Connection Tests (NCT) A and B and Digit Symbol were available in 44 unimpaired patients, 6 patients with minimal hepatic encephalopathy (HE), and 25 patients with overt HE. Significant differences between patients with minimal and patients with overt HE: cc p < 0.01.
to the International 10–20 system (Walter-Graphitec system equipment). The traces underwent spectral analysis and were classified according to Amodio et al. (2), with a modified slow theta activity threshold of 30% (38).

Neuropsychiatric status was classified as 1) unimpaired: no clinical evidence of hepatic encephalopathy and no defining EEG or psychometric abnormalities; 2) minimal hepatic encephalopathy: no clinical evidence of hepatic encephalopathy but abnormal EEG and/or impaired psychometric performance; and 3) overt hepatic encephalopathy: clinically evident neuropsychiatric disturbances.

Assessment of HRV

Data acquisition. A 10-min, single channel electrocardiogram (ECG) was recorded simultaneously with the ECG by placing a silver-silver chloride electrode on each wrist (WG PLEEG system). The ECG data were exported at a sampling rate of 256 Hz. The R peaks were detected and the R-R interval series was generated by using an ad hoc computer program. The R-R interval series was visually inspected and 5-min, artifact-free continuous R-R interval sections were selected for analysis (53).

Linear analysis of HRV. Linear measures of HRV provide information on the degree of variability in the R-R time series. The standard deviation of the R-R intervals (SDNN) was calculated on the selected artifact-free trace and used as a measure of total HRV. Spectral analysis of the R-R interval time series was carried out by fast Fourier transformation on 1,024 sample points, by applying Welch’s window, with software developed by Niskanen et al. (41). Two bands were identified: 1) a low-frequency component (LF: 0.04–0.15 Hz), which reflects the oscillatory pattern of the baroreflex loop and is jointly mediated by sympathetic and parasympathetic activities (1), and 2) a high-frequency component (HF: 0.15–0.4 Hz), which reflects the inhibition of vagal tone during inspiration (1). The LF/HF ratio was used as a measure of sympathovagal balance (1).

Nonlinear analysis of HRV. Nonlinear measures of HRV provide information on the structure or complexity of the R-R time series. In the present study nonlinear measures of HRV was assessed using Poincaré plot as well as sample entropy analysis.

POINCARE PLOT. The Poincaré plot is a graphical representation of the correlation between consecutive R-R intervals [x-axis: R-R (n); y-axis: R-R (n+1); Fig. 1]. If the cardiac rhythm is regular, the points in the Poincaré plot will be located close to the line of identity. The standard deviation of the points perpendicular to the line of identity (SD1) describes short-term variability, which is mainly related to the effects of respiration on vagal drive (56); the standard deviation along the line of identity (SD2) describes the long-term R-R interval variations and accounts for all other heart rate changes, including those associated with sympathetic oscillations, baroreflex loop, thermoregulation, and fluctuations in humoral factors (56). The parameters SD1 and SD2 were calculated by using software developed by Niskanen et al. (41).

SAMPLE ENTROPY. The sample entropy (SampEn) quantifies the degree of regularity vs. the degree of unpredictability of a time series (49). SampEn is the logarithmic likelihood of the repetition of patterns in the time series; it calculates the probability that an epoch of window length m, with a degree of tolerance r, will be repeated at later time points. Regular time series are characterized by low SampEn, whereas random time series are characterized by high SampEn. In the present study, m was fixed at 2 and r at 0.2 ms (49).

Measurement of Plasma Cytokine Concentration

Plasma samples were collected from the last 18 patients with cirrhosis enrolled in the study and were stored at −80°C until analyzed for TNF-α, IL-6, IL-10, and IL-12 by using eBioscience ELISA kits (San Diego, CA) on Nunc Maxisorb 96-well ELISA plates (Nunc, Roskilde, Denmark), according to the manufacturers’ instructions. The lower detection limit for the assays was 3 pg/mL. The

<table>
<thead>
<tr>
<th>Table 2. Indexes of HRV in healthy volunteers and in patients with cirrhosis, by degree of hepatic dysfunction and neuropsychiatric status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Populations</td>
</tr>
<tr>
<td>Healthy volunteers (n=11)</td>
</tr>
<tr>
<td>Patients with cirrhosis (n=80)</td>
</tr>
<tr>
<td>Child A (n=51)</td>
</tr>
<tr>
<td>Child B (n=16)</td>
</tr>
<tr>
<td>Unimpaired (n=44)</td>
</tr>
<tr>
<td>Minimal HE (n=9)</td>
</tr>
<tr>
<td>Overt HE (n=9)</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; data for sample entropy (SampEn) were available in 10 healthy volunteers. HRV, heart rate variability; HR, heart rate; SD1, short-term R-R variability; SD2, long-term R-R variability; HE, hepatic encephalopathy; HE1, minimal HE; HE2, overt HE; HE1/HE2, HE1/HE2.

**P**< 0.05 vs. HE1; **P**< 0.05 vs. HE2; **P**< 0.01 vs. HE1; **P**< 0.01 vs. HE2.

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intra-assay coefficients of variation for TNF-α, IL-6, IL-10, and IL-12 were 2.9, 3.9, 1.2, and 1.3%, respectively.

Statistical Analysis

Differences between normally distributed variables were examined by the Student’s t-test or by one-way ANOVA; post hoc analyses were performed by Tukey’s test. Differences between nonnormally distributed variables were examined by the Mann-Whitney U-test or by Kruskal-Wallis ANOVA; post hoc analyses were performed by using Dunn’s test. The relationship between HRV and neuropsychiatric impairment was examined by analysis of covariance, adjusted for the degree of liver failure and vagal modulation. Correlations between variables were tested by using the Spearman R coefficient of correlation. Patients were followed prospectively for a mean (range) of 20.3 (1.0–44.9) mo from the date of initial assessment. The relationship between HRV indexes and survival was examined by Cox’s proportional hazards model. Analyses were undertaken using Statistica 6.0 (StatSoft, Tulsa, OK), GraphPad Prism (version 3.03, GraphPad Software, 1994–2002, San Diego, CA), and Stata (release 9.1, StataCorp, 1984–2005, College Station, TX) statistical packages.

Ethics

This study was conducted according to the Declaration of Helsinki (Hong Kong Amendment) and Good Clinical Practice (European guidelines). The protocol was approved the Royal Free Hampstead National Health Service Trust Ethics Committee. All participating subjects provided written, informed consent.

RESULTS

All 80 patients were clinically stable at the time of assessment; 66 (83%) were assessed as outpatients. The etiology of the cirrhosis was alcohol in 65 (81%), chronic viral hepatitis (hepatitis B or C virus) in seven (9%), and “various” in the remaining eight (10%). Functionally, 51 (64%) of the 80 patients were classified as Child’s grade A, 13 (16%) as Child’s grade B, and 16 (20%) as Child’s grade C. Four of the patients with overt hepatic encephalopathy were incapable of performing the psychometric tests. One clinically unimpaired patient was noncompliant; he was classified as having minimal hepatic encephalopathy on the basis of an abnormal EEG. Thus, on the day of study, 44 (55%) of the 80 patients were classified as neuropsychiatriically unimpaired, seven (9%) as having minimal, and 29 (36%) as having overt hepatic encephalopathy (Table 1). The patients with alcohol-related cirrhosis had been abstinent from alcohol for a mean (range) of 34 (3–360) mo. None of the patients had historical, clinical, or ECG evidence of intrinsic or ischemic heart disease.

HRV Assessments

The patients with cirrhosis had a significantly higher mean heart rate than the healthy controls, although this increase in rate was confined to the patients with the most compromised liver disease (Table 2). The mean HRV was significantly decreased, in the patient population, reflecting reductions in both the variability of the R-R time series (linear measures: SDNN, \( P < 0.001 \); LF, \( P < 0.001 \); HF, \( P < 0.01 \)) and its complexity (nonlinear measures: SD1, \( P < 0.01 \); SD2, \( P < 0.001 \); SampEn, \( P < 0.01 \)) (Table 2). No significant differences in HRV were observed between patients with alcohol-related cirrhosis and patients with cirrhosis of other etiologies.

In the patients with cirrhosis, mean HRV indexes decreased in parallel with the degree of hepatic dysfunction (Table 2).
indexes of both R-R variability [SDNN (observed between the grade of hepatic encephalopathy and psychiatric impairment. Thus significant correlations were again indexes also decreased in parallel with the degree of neuropsychiatric grade A and Child’s grade C disease (Table 2). Mean HRV HRV variables were observed between patients with Child’s no longer significant when adjusted for the degree of neuropsychiatric impairment [hepatic encephalopathy (HE)] and the degree of hepatic dysfunction (analysis of covariance, Fig. 3). Relationship between decreasing SD2 and deteriorating hepatic function was independent of the degree of hepatic dysfunction [F(2,76) = 4.8; P = 0.011; Figs. 2 and 3A], the relationship between SD2 and the degree of hepatic dysfunction was lost when adjusted for the degree of neuropsychiatric impairment [F(2,76) = 0.3; P = 0.73; Fig. 3B].

The association between SD2 and neuropsychiatric impairment held firm even when controls were exercised for indicators of vagal modulation, viz., the linear spectral high-frequency component, HF, which reflects the inhibition of vagal tone during inspiration [F(2,76) = 6.7; P = 0.002; covariate mean: 56.6] and the nonlinear variable SD1, which mainly reflects the effects of respiration on vagal drive [F(2,76) = 5.7; P = 0.005; covariate mean: 12.3] (Table 3).

Significant correlations were observed between HRV indexes and individual psychometric and spectral EEG variables (Table 4).

**Plasma Cytokines**

Plasma cytokine concentrations were below the assays’ levels of detection in the healthy volunteers. In the patients with cirrhosis, significant concentrations of all the measured cytokines were detected, viz., TNF-α: 12.1 ± 21.6 pg/ml; IL-12: 6.8 ± 1.73 pg/ml; IL-10: 9.0 ± 13.3 pg/ml, and IL-6: 5.1 ± 3.35 pg/ml.

Significant correlations were observed between plasma IL-6 concentrations and both linear and nonlinear HRV indexes (Table 5). Similarly, significant correlations were observed between cytokine plasma concentrations and psychometric test variables (Table 5).

**Survival**

Patients were followed prospectively for a mean (range) of 20.3 (10.4-44.9) mo from the date of initial assessment. Nine patients died during the follow-up period and two underwent orthotopic liver transplantation. There was a significant relationship between SD2 and survival (P = 0.01); the relative risk of death increased by 7.7% (95% confidence interval, 1.8-13.6) for every 1-ms drop in this variable.

**DISCUSSION**

HRV decreased significantly in the patients with cirrhosis, in the present study, in parallel with their degree of neuropsychiatrically impaired patients and patients with overt hepatic encephalopathy (Table 2).

Thus HRV, which is best represented by the nonlinear variable SD2, decreased significantly in parallel with both deterioration in hepatic function and deterioration in neuropsychiatric status. However, whereas the relationship between SD2 and the degree of neuropsychiatric impairment was independent of the degree of hepatic dysfunction [F(2,76) = 4.8; P = 0.011; Figs. 2 and 3A], the relationship between SD2 and the degree of hepatic dysfunction was lost when adjusted for the degree of neuropsychiatric impairment [F(2,76) = 0.3; P = 0.73; Fig. 3B].

<table>
<thead>
<tr>
<th>HRV Parameter</th>
<th>Correction Variable</th>
<th>Covariate Mean</th>
<th>Unimpaired</th>
<th>Minimal HE</th>
<th>Overt HE</th>
<th>F(2,76)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD2</td>
<td>HF</td>
<td>56.6 (ms²)</td>
<td>37.3 ± 1.8</td>
<td>35.8 ± 4.4</td>
<td>26.8 ± 2.2</td>
<td>6.7</td>
<td>0.002</td>
</tr>
<tr>
<td>SD2</td>
<td>SD1</td>
<td>12.3 (ms)</td>
<td>36.7 ± 1.6</td>
<td>35.0 ± 3.9</td>
<td>27.9 ± 2.0</td>
<td>5.7</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Data are expressed as covariate-adjusted means ± SE.

**Figure 3.** Analysis of long-term HRV (mean SD2) in patients with cirrhosis, adjusted for neuropsychiatric status [hepatic encephalopathy (HE)] and the degree of hepatic dysfunction (Child’s grade). A: relationship between decreasing SD2 and deteriorating neuropsychiatric status was maintained after adjustment for the degree of hepatic dysfunction (analysis of covariance, P = 0.011). B: relationship between decreasing SD2 and deteriorating hepatic function was no longer significant when adjusted for the degree of neuropsychiatric impairment (analysis of covariance, P = 0.73).
Psychometric performance given that appear to have been considered previously. This is surprising with cirrhosis might relate to the changes in neuropsychiatric linear interactions within the system.

identified a complex, multifaceted disturbance, involving non-investigation of the disturbed process was undertaken that 37, 39). However, in the present study a much more detailed (37), or, more frequently, sympathovagal imbalance (6, 12, 32, 37), sympathetic hyperfunction (17, 25, 37), sympathetic hypofunction (17, 25, 37, 39). In the present study, HRV indexes (6, 12, 17, 25, 32, 37, 39). However, in the majority of these 32, 37, 39). In the present study, HRV indexes decreased in parallel with the degree of hepatic dysfunction but this relationship was lost when adjusted for the degree of neuropsychiatric impairment.

The possibility that the changes in HRV observed in patients with cirrhosis might relate to the changes in neuropsychiatric status commonly observed in this patient population does not appear to have been considered previously. This is surprising given that 1) reductions in HRV are known to occur in a number of neuropsychiatric disorders (28, 29, 58, 62) and 2) Miyajima and colleagues (37) reported a highly significant relationship between decreased HRV and gastric dysmotility in patients with cirrhosis and a positive correlation between gastric motility and the degree of hepatic encephalopathy.

There are several possible explanations for the relationship between HRV and neuropsychiatric status observed in these patients. The two most likely are 1) the presence of an autonomic neuropathy or 2) the presence of a mechanism involving circulating inflammatory cytokines.

Vagal neuropathy is common in patients with cirrhosis and is an independent predictor of mortality (17, 25). Its presence may lead to prolongation of gastrointestinal transit time, small bowel bacterial overgrowth, bacterial translocation and endotoxemia, all of which may predispose to hepatic encephalopathy. Indeed, Maheshwari and colleagues (33) reported that patients with cirrhosis and evidence of an autonomic neuropathy are more likely to develop hepatic encephalopathy than those without. They did not, however, objectively quantify their patients’ neuropsychiatric status.

Many of the measures of HRV obtained by using nonlinear dynamics, such as SD2 and SampEn, are conceptually complete but can be optimized to provide useful information to clinicians. In the present study, Poincaré plots were used to distinguish the effects of vagal modulation from other causes of heart rate variation (56). In these plots, the variable SD1 reflects short-term R-R interval variations, which mainly relate to vagal drive (56), whereas the variable SD2 reflects long-term R-R interval variations and reflects all the other causes of heart rate change, e.g., those associated with sympathetic oscillations, the baroreflex loop, thermoregulation, and fluctuations in humoral factors (56). The heart rate variable that showed the strongest correlation with neuropsychiatric status was SD2, and this relationship held firm even when control was exercised for vagal drive. This indicates that the relationship between HRV and hepatic encephalopathy cannot be attributed simply to vagal dysfunction.

Decreased HRV has been reported in a variety of clinical settings associated with increased production of inflammatory cytokines (6, 22–24, 31). Circulating levels of inflammatory cytokines are increased in patient with cirrhosis (19, 55) and positively correlate with the severity of hepatic encephalopathy in this patient population (19, 43, 44). In the present study, plasma IL-6 concentrations correlated significantly with both indexes of HRV and neuropsychiatric performance, suggesting that these changes share a common etiology. These data also provide support for the potential role of inflammatory mediators in the pathogenesis of hepatic encephalopathy (27, 42, 51).

The physiological mechanism underlying the loss of HRV during the systemic inflammatory response is unknown but cytokine-induced autonomic dysfunction or disruption of intracellular signal transduction processes undoubtedly play a

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR-Mean</th>
<th>SDNN</th>
<th>SD1</th>
<th>SD2</th>
<th>LF</th>
<th>HF</th>
<th>LF/HF</th>
<th>SampEn</th>
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<tr>
<td>NCT-A, s</td>
<td>0.03</td>
<td>−0.26*</td>
<td>−0.19</td>
<td>−0.27*</td>
<td>−0.22</td>
<td>−0.19</td>
<td>−0.02</td>
<td>−0.20</td>
</tr>
<tr>
<td>NCT-B, s</td>
<td>0.18</td>
<td>−0.33*</td>
<td>−0.28*</td>
<td>−0.35**</td>
<td>−0.29*</td>
<td>−0.30**</td>
<td>0.13</td>
<td>−0.31**</td>
</tr>
<tr>
<td>DS, n correct</td>
<td>−0.11</td>
<td>0.30**</td>
<td>0.25*</td>
<td>0.32**</td>
<td>0.27*</td>
<td>0.24*</td>
<td>−0.03</td>
<td>0.26*</td>
</tr>
<tr>
<td>MDF, Hz</td>
<td>0.02</td>
<td>0.22*</td>
<td>0.15</td>
<td>0.21</td>
<td>0.20</td>
<td>0.20</td>
<td>−0.04</td>
<td>0.18</td>
</tr>
<tr>
<td>Theta power, %</td>
<td>0.04</td>
<td>−0.27*</td>
<td>−0.20</td>
<td>−0.26*</td>
<td>−0.25*</td>
<td>−0.23*</td>
<td>0.05</td>
<td>−0.19</td>
</tr>
</tbody>
</table>

Values are correlation coefficients; *P < 0.05; **P < 0.01. §Data for NCT-A and -B and for Digit Symbol (DS) were available in 44 unimpaired patients with cirrhosis, 6 with minimal HE, and 25 with overt HE.
and thus it has been suggested that overexpression of cytokines can potentially blunt β-adrenergic signaling (47), and thus it has been suggested that overexpression of cytokines and subsequent loss of β-adrenergic responsiveness might contribute to the decrease in HRV during inflammation (34). Although this hypothesis is attractive within the context of “cirrhotic cardiomyopathy” (18, 61), recent studies have shown that decreased HRV following endotoxin challenge is not related to alterations in cardiac β-adrenergic signaling in endotoxemic mice (36). In addition, loss of HRV in a rat model of cirrhosis occurs independently of any impairment in cardiac β-adrenergic responsiveness (35).

Although the brain used to be considered an “immune-privileged” organ, it is now thought to monitor peripheral innate immune responses by several mechanisms involving parallel pathways (16). These include (1) primary afferent neurons, such as the vagus nerve, which carries information from the viscera; (2) Toll-like receptors on macrophage-like cells residing in the periventricular regions; (3) cytokine receptors located on the endothelial cells of brain venules; and (4) cytokine transporters at the blood brain barrier level (16). Within this frame, a reciprocal relationship between hepatic and central nervous system (CNS) inflammatory responses has been postulated (8–10). Pioneering studies by Campbell and colleagues (8–10) have shown that the liver plays a putative role in the CNS inflammatory response. Thus selective depletion of hepatic Kupffer cells or hepatic nuclear factor-κB, a proinflammatory transcription factor, attenuate the inflammatory response in experimental models of cytokine-induced brain injury (9, 10). On the basis of these observations, it is possible to postulate that hepatic inflammation may potentiate the effect of cytokines on the brain. Such an interaction between neural and hepatic inflammatory responses might also play a mechanistic role in the development of hepatic encephalopathy.

Cytokine-induced neural modulation can affect the brain cortex as well as subcortical regions such as the medullary centers (16). There is convincing evidence that the central cardiovascular medullary center is disordered in experimental cirrhosis (52), which may result in uncoupling of the cardiac pacemaker cells from their brain stem regulators. Following from Pincus’s reasoning that uncoupling and increased “system isolation” are associated with greater regularity (46), medullary center dysfunction may be implicated in the regularization of the cardiac cycle during systemic inflammation.

The present study shows a relationship between inflammatory markers, neuropsychiatric impairment, and decreased HRV in patients with hepatic encephalopathy. These findings are in agreement with a recent report by Newton et al. (40), who showed that cognitive symptoms in patients with primary biliary cirrhosis are independent of liver disease severity and are associated with autonomic dysfunction. The correlation between neuropsychiatric status and HRV observed in the present study suggests a common pathogenic mechanism mediated by cytokine-induced changes in the cortical and medullary brain regions involved in cardiovascular control.

There is also some evidence, from previous studies, that decreased HRV is an independent risk factor for death and thus has a negative prognostic value in this patient population (6, 25). This finding was confirmed in the present study; the relative risk of death increased by 7.7% for every 1-ms drop in SD2. Thus, whatever the causal link between changes in HRV and neuropsychiatric status in patients with hepatic encephalopathy, a reduction in HRV identifies individuals who at risk of death and this variable could be used to monitor patients over time and perhaps facilitate selection for transplantation.

Limitations of the Study

A potential limitation of the present study is that a significant proportion of the patients had alcohol-related cirrhosis. However, since they had been abstinent from alcohol for a minimum of 3 mo and, in most cases, for years, the observed relationship between HRV and hepatic encephalopathy is unlikely to be related to the acute effects of ethanol on the cardiovascular and/or the nervous system. In addition, frozen plasma samples were not available for all patients enrolled in the study. Although the presented correlation between plasma IL-6 levels and HRV indexes observed in the 18 patients with available data is promising, an IL-6 threshold value to predict hepatic encephalopathy or decreased HRV could not be identified, most likely because of insufficient study power.

Larger studies in patients with cirrhosis of varying etiology and more complete cytokine data analysis should be carried out in the future to confirm our findings and further clarify the relationship between cardiovascular variability, neuropsychiatric impairment, and systemic inflammation.

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