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CIRRHOSIS AND LIVER FAILURE

Serum nitrotyrosine and psychometric tests as indicators of impaired fitness to drive in cirrhotic patients with minimal hepatic encephalopathy

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Abstract

Background & Aims: Cirrhotic patients with minimal hepatic encephalopathy (MHE) show impaired driving ability and increased vehicle accidents. The neurological deficits contributing to impair driving and the underlying mechanisms are poorly understood. Early detection of driving impairment would help to reduce traffic accidents in MHE patients. It would be therefore useful to have psychometric or biochemical parameters reflecting driving impairment. The aims of this work were as follows: (i) to shed light on the neurological deficits contributing to impair driving; (ii) to assess whether some psychometric test or biochemical parameter is a good indicator of driving impairment. *Methods:* We assessed in 22 controls, 36 cirrhotic patients without and 15 with MHE, driving performance using a driving simulator (SIMUVEG) and Driver Test. MHE was diagnosed using the psychometric hepatic encephalopathy score (PHES). Psychometric tests assessing different neurological functions (mental processing speed, attention, visuo-spatial and bimanual coordination) were performed. Blood ammonia and parameters related with nitric oxide-cGMP metabolism, IL-6, IL-18 and 3-nitrotyrosine were measured. *Results:* Patients with MHE showed impaired driving ability correlating with MHE grade, with impaired vehicle lateral control in spite of reduced driving speed. Patients with MHE show psychomotor slowing, longer reaction times, impaired bimanual and visuo-spatial coordination and concentrated attention and slowed speed of anticipation and increased blood ammonia, cGMP, IL-6, IL-18 and 3-nitrotyrosine. *Conclusions:* Impaired mental processing speed, attention and alterations in visuo-spatial and motor coordination seem main contributors to impaired driving ability in patients with MHE. Increased serum 3-nitrotyrosine is associated with impaired driving ability.

Patients with cirrhosis may present minimal hepatic encephalopathy (MHE), showing intellectual and motor alterations (1–3). MHE is associated with falls and poor quality of life (4, 5). Although a pioneer study from Blei and coworkers (6) did not find deficiencies in simulated or real driving performance in patients with MHE, subsequent studies have shown impaired fitness to drive both in on-road driving tests (7, 8) and in driving simulators (9), with impaired navigation skills (10) and a greater frequency of motor vehicle accidents (11, 12). It has been proposed that treatment of MHE could substantially reduce societal costs by preventing motor vehicle accidents (13).

The neurological deficits (mental processing speed, attention, visuo-spatial coordination…) that contribute to impair driving in MHE and the underlying molecular mechanisms are poorly understood. Impairment in attention and in response inhibition seems to contribute to driving impairment (10). Patients suffering from MHE present a variety of mild alterations including psychomotor slowing and mild cognitive impairment with attention deficit and alterations in visuo-motor coordination and working memory (14–17), which could contribute to impair driving ability in patients with MHE.

The aims of this study were as follows:

(i) to shed light on which cognitive, motor or functional deficits contribute to impair driving in MHE patients.

(ii) to assess whether some psychometric test or biochemical parameter is a good indicator of driving impairment.

We performed psychometric tests to assess the relative contribution of different functional alterations (mental processing speed, attention, visuo-spatial coordination, tendency to impulsivity, bimanual coordination, fatigue potential…) to the impairment of driving ability.

To look for biochemical parameters reflecting driving impairment in patients with MHE, we measured several parameters that have been associated with neurological impairment in MHE. Hyperammonaemia and inflammation are main contributors to mild cognitive impairment in MHE (18–22). MHE correlates with increased levels of the pro-inflammatory cytokines IL-6 and IL-18 and with altered nitric oxide (NO)-cGMP homeostasis and increased activation of soluble guanylate cyclase by NO in freshly isolated lymphocytes (22, 23). Serum level of 3-Nitrotyrosine is a good predictor of the presence of MHE in patients with cirrhosis (24). Taking into account these reports, we assessed the possible utility of ammonia, cGMP, NO metabolites, activation of guanylate cyclase by NO, IL-6, IL-18 or 3-nitrotyrosine as biomarkers for driving impairment in patients with MHE.

Patients and methods

Patients with cirrhosis and controls

Fifty-six patients with liver disease and 25 controls were enrolled in this study after written informed consent was obtained. Inclusion criteria: patients were included if they had clinical, biochemical and histological evidence of hepatic cirrhosis caused by alcoholic liver disease. This study also included control subjects for whom liver disease was discarded by clinical, analytical, serological and echographic analysis. All subjects had valid driver's licence.

Exclusion criteria: Patients were excluded if they had clinical evidence of overt HE, decompensate diabetes with high levels of glycosylated haemoglobin, renal dysfunction, hyponatraemia, concomitant neurological disease, severe cardiovascular disease or antibiotic use. Patients had to be abstinent from alcohol for at least 6 months prior to this study to stabilize the disease. Eight subjects (3 controls and 5 patients) were excluded from this study by problems of "simulator sickness." The composition of the groups, the number of subjects, age and analytical data are given in Table 1. After a standard history and physical examination, blood was drawn for routine laboratory measures (Table 1). This study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (25) and was approved by the Scientific and Ethical Committees of the Hospital Clinico Universitario de Valencia, on May 27, 2010. Psychometric tests, determination of CFF (Table 1) and blood collection were carried out on the same day, and driving tests in the simulator were performed within the same week.

Obtention of plasma and serum

Blood (5 ml) was taken in BD Vacutainer tubes with or without EDTA (for plasma and serum respectively) and centrifuged at 500 *g* for 10 min. The supernatant was collected, and stored frozen at −80°C in aliquots of 500 μl.

Biochemical determinations in blood

The stable metabolites of NO (nitrates+nitrites) were measured as nitrite after enzymatic conversion of nitrate by nitrate reductase, as previously described (26). Interleukins IL-6 and IL-18 were determined in serum using the BIOTRAK Easy ELISA system from Amersham Biosciences UK, Ltd. cGMP in plasma was measured using the BIOTRAK™ cGMP enzyme immunoassay kit from Amersham (GE Healthcare, Life Sciences, Buckinghamshire, UK). Ammonia in blood was measured as described previously (23, 26). 3-nitrotyrosine was measured by high-pressure liquid chromatography as in (24).

Isolation of lymphocytes and activation of soluble guanylate cyclase

Activation of soluble guanylate cyclase by the nitric oxide-generating agent S-nitroso-N-acetylpenicillamine (SNAP) (Molecular Probes, Eugene, OR, USA) in intact lymphocytes was analysed as in (23) and was calculated as the ratio between cGMP in lymphocytes after and before incubation with SNAP.

Assessment of MHE with the PHES battery of psychometric tests

MHE was diagnosed using the PHES that has been recommended as the "gold standard" in the diagnosis of MHE (27). This battery comprises five psychometric tests: the digit symbol test (DST), the number connection test A (NCT-A), the number connection test B (NCT-B), the serial dotting test (SD) and the line tracing test (LTT). DST test evaluates processing speed and working memory, NCT-A and NCT-B are tests of mental processing speed and attention, and SD and LTT are related to visuo-spatial coordination. Subjects performed the five tests together with the measurement of the CFF in the same session. The PHES was calculated adjusting for age and education level by means of Spanish normality tables that are freely available (www.redeh.org), and patients were classified as having MHE when the score was less than −4 points.

Critical flicker frequency

The CFF was measured in a quiet, semidarkened room without distracting noises using a portable, battery powered analyser (Hepatonorm™ Analyzer; R&R Medi-Business Freiburg GmbH, Freiburg, Germany), as previously described (28, 29). CFF was not considered

Table 1. Characteristics and analytical data of the different groups

	Range	Control	Patients without MHE	Patients with MHE
Total individuals		22	36	15
Age		51 ± 16	54 ± 10	63 ± 10
Ascites (number of patients)		–	5	3
Child Pugh A/B/C		–	32/4/0	10/5/0
MELD		–	8.3 ± 2	9.3 ± 2
PHES		0.22 ± 0.13	−0.4 ± 0.2	−6.4 ± 0.7[c,***]
Digit symbol test		−0.09 ± 0.06	−0.08 ± 0.1	−0.67 ± 0.23
Number connection test A		0.0 ± 0.0	0.03 ± 0.05	−1.33 ± 0.29[b,**]
Number connection test B		0.14 ± 0.07	0.0 ± 0.09	−1.93 ± 0.27[c,***]
Serial dotting test		0.0 ± 0.0	−0.25 ± 0.08[a]	−1.40 ± 0.33[b,*]
Line tracing test		0.18 ± 0.07	0.11 ± 0.09	−1.07 ± 0.27[b,**]
CFF (Hz)		42.7 ± 0.5	41 ± 0.4	39 ± 0.5[c,**]
AST (mU/ml)	1–37	20 ± 4.0	73 ± 56[c]	82.5 ± 58[c]
ALT (mU/ml)	1–41	18 ± 6.0	77 ± 24[c]	90.1 ± 24[c]
GGT (mU/ml)	10–49	26.7 ± 5	86.4 ± 60[c]	106 ± 64[c]
Uric acid (mg/dl)	2.5–7	4.0 ± 1.0	6.2 ± 2.0	5.73 ± 2.3
Creatinine (mg/dl)	0.5–1.3	0.92 ± 0.1	1.1 ± 0.2	1.2 ± 0.2
Cholesterol (mg/dl)	140–200	172 ± 22	175 ± 44	167 ± 55
Triglycerides (mg/dl)	40–160	95 ± 32	111 ± 64	119 ± 64
Bilirubin (mg/dl)	0.1–1	0.6 ± 0.2	1.7 ± 0.7[c]	2.3 ± 0.6[c]
Albumin (g/dl)	3.5–5	4.4 ± 0.2	3.7 ± 0.6[c]	2.9 ± 0.6[c,*]
Prothrombin time (s)		13 ± 1.3	24 ± 4[c]	30 ± 4[c]
Fibrinogen (g/l)	2–4	3.1 ± 1.0	3.3 ± 1.3	3.6 ± 1.2
Alkaline phosphatase (mU/ml)	50–250	147 ± 53	216 ± 77[b]	314 ± 96[c,*]
Erythrocytes	4.2–6.1	4.6 ± 0.4	4.3 ± 0.7	3.4 ± 0.6
Leucocytes	4.8–10.8	6.5 ± 1.3	6 ± 2.6	5.5 ± 2.0
Neutrophils (%)	55–75	55 ± 7.4	54 ± 6.2	59 ± 9.3
Lymphocytes (%)	17–45	35 ± 6.0	29 ± 10	27 ± 9.4
Monocytes (%)	2–8	6.0 ± 1.3	8.4 ± 3.0[b]	10 ± 2.6[c]
Eosinophils (%)	1–4	3.3 ± 2.0	2.4 ± 1.2	1.7 ± 1.0
Basophils (%)	0.05–0.5	0.5 ± 0.2	0.6 ± 0.3	0.6 ± 0.1

Values are expressed as mean ± SEM for CFF, PHES and PHES subtests, and as mean ± SD for MELD and analytical data. Values that are significantly different from controls are indicated by superscripts: [a]$P \leq 0.05$; [b]$P < 0.01$; [c]$P < 0.001$. Values that are significantly different in patients with and without MHE are indicated by *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

CFF, Critical Flicker Frequency; PHES, Psychometric Hepatic Encephalopathy Score; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl-transpeptidase.

in subjects with visual defects (6 patients without and 2 with MHE).

Assessment of driving ability in the SIMUVEG driving simulator

A good procedure to evaluate driving performance is the use of driving simulation (30). We used the SIMUVEG driving simulator, a Computer System for Driver Interactive Evaluation designed by INTRAS (Institute of Traffic and Road Safety, Valencia University, Spain) and consisting of a Renault Twingo, without engine, but complete set of controls, a PC, sending driving data from the car to a Silicon Graphics ONYX2 Infinity Reality workstation that able to generate all the images needed to display the driving simulation scenes in real-time, and a 120 degrees screen projection system providing a complete field of view in front of the vehicle. Inside SIMUVEG, driving assessment was done by means of driving scenarios in which the subject had to drive for 20 min. A large quantity of variables related with paths, times, distances, actions and decisions were measured in each scenario that allowed assessing the driving style of the drivers. Two essential aspects of driving performance are the longitudinal control and the lateral control of the vehicle (31). Longitudinal control is related with the average speed. Lateral control is related with the angular speed, the wheel swerving, the distance to line crossing and the time to line crossing. All these measures are related and for this study, we focus on the average speed and the time to line crossing (TLC). Proper lateral control of the vehicle involves following a parallel path to the lines of the road. TLC can be computed using the procedure described in van Winsum *et al.* (32). We evaluated if at least one episode of low TLC happens in sections of 10 m of the circuit and then the percentage of sections with low TLC per individual was computed. The resulting measure is called the MINTL in which high values, close to 100,

are equivalent to poor driving and low values, close to 0, mean excellent driving. The threshold for low TLC was set at 1.5 s.

Classification of subjects as good or bad drivers

Subjects were classified as good or bad drivers (keeping or not driving abilities) according to the performance in the driving simulator. The cut-off for MINTL or speed parameters was considered as the mean value for control subjects ± 2 standard deviations. The cut-off for MINTL was 30 and for speed 58.6 km/h. Subjects with MINTL greater than 30 and/or speed lower than 58.6 km/h were considered as bad drivers.

Assessment of driving skills using psychometric tests

The Basic Skills Assessment of the Driver Test Battery (33), used in Spain to renew the driving licence, was used. The battery consists of four basic tests evaluating the following areas of subject skills:

Speed of anticipation

The subject ability to judge distance/speed and possible tendencies towards impulsivity are assessed. The parameters measured are as follows: MDT, Mean Deviation Time: absolute average of deviations in time (in seconds). MDD, Mean Deviation Distance: absolute average of deviations in distance (in pixels).

Bimanual coordination

The subject's task is to coordinate and dissociate movements of each hand while interacting with continuously moving stimuli. The subject's ability to simultaneously coordinate separate tasks and the extent to which he/she is able of correcting or modifying their reactions to achieve an appropriate result are evaluated. The parameters measured are as follows: EP, error percentage over the total trajectory; TT, total time of error with both hands (in seconds); TN, total number of errors with both hands.

Multiple discriminative reactions

The subject is required to provide numerous motor responses using hands and feet when faced with a series of visual stimuli and sounds. The ability to discriminate the correct stimuli and correct allocation of responses to stimuli faced is evaluated. This test evaluates subject's ability to act appropriately when faced with specific situations, referring to basic decision-making ability. The parameters measured are as follows: MRT, mean reaction time of right and wrong answers (in seconds); RA, number of right answers; ER, number of errors; NA, number of not answered stimuli.

Concentrated attention and resistance to monotony

Similar to the previous test, the stimuli are given in a fixed sequence. This test evaluates the subject's appropriate behaviour in repetitive, monotonous driving conditions. The level of potential fatigue is also evaluated. The parameters measured are as follows: MRT, RA, ER and NA, as in the previous test.

Statistical analysis

All values are given as mean ± standard error (SEM). Variables were compared between groups using univariate analysis of covariance (ANCOVA) with age included as covariate, followed by post-hoc Bonferroni. The probability level accepted for significance was $P < 0.05$. Bivariate correlations among variables were evaluated using the Pearson correlation test. Univariate and multivariate logistic regressions were performed using driving ability (according to simulator parameters) as dependent variable. Potential explanatory variables used on univariate analysis were those showing significant ($P < 0.05$) differences between cirrhotic patients classified as good or bad drivers (according to simulator parameters). Multivariate logistic regression analysis was performed including both psychometric and biochemical parameters (those that were significant on univariate analysis) as independent variables. Data were processed using the software package SPSS Version 17.0 (SPSS Inc, Chicago, IL, USA).

Results

Fifteen of the 51 (29%) cirrhotic patients included in this study showed MHE as determined using the PHES battery of psychometric tests. The PHES was lower ($P < 0.001$) in patients with MHE than in those without MHE. It was not different in patients without MHE and control subjects (Table 1). CFF was lower in patients with MHE than in patients without MHE ($P < 0.01$) (Table 1). There was a good correlation ($r = 0.470$, $P < 0.001$) between the performance in the PHES battery and the CFF.

Driving ability is impaired in patients with MHE

Driving ability in the SIMUVEG driving simulator was impaired in cirrhotic patients with MHE, but not in those without MHE as assessed by measuring average driving speed (Fig. 1A) and lateral control of the vehicle (MINTL) (Fig. 1B). Driving speed was not different in controls (80 ± 2 km/h) and in cirrhotic patients without MHE (77 ± 1 km/h), but was reduced (60 ± 2 km/h, $P < 0.001$) in patients with MHE (Fig. 1A).

The lateral control of the vehicle was not affected in cirrhotic patients without MHE. These patients showed a MINTL of 20 ± 1, not different from that of controls (17 ± 1). Lateral control was impaired ($P < 0.003$) in patients with MHE, who showed a MINTL of 27 ± 4 (Fig. 1B).

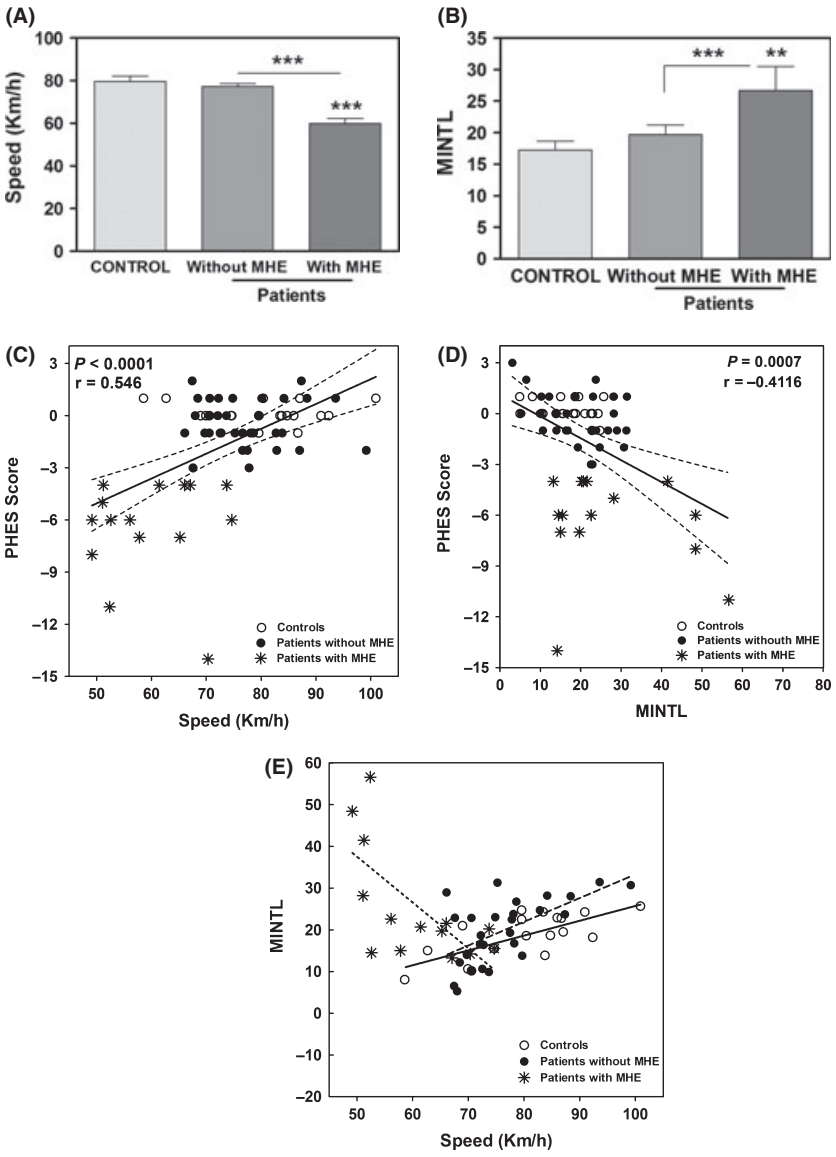

Fig. 1. Patients with minimal hepatic encephalopathy (MHE) show impaired driving ability, with reduced speed and lateral control of the vehicle (MINTL) correlating with MHE. MHE patients showed slower speed (A) and higher MINTL values (B) in the driving simulator than controls and patients without MHE. There were good correlations between the performance in the PHES battery and speed ($P < 0.0001$, $r = 0.546$) (shown in C) and MINTL ($P = 0.0007$, $r = -0.416$) (shown in D). (E) Shows the relationship between MINTL and speed for each group of subjects. There was a positive correlation between these parameters in controls and patients without MHE ($r = 0.707$, $P = 0.0007$, and $r = 0.607$, $P = 0.0008$, respectively), but patients with MHE had a negative relationship ($r = -0.682$, $P = 0.005$). Values are the mean ± SEM of 22 controls, 36 patients without and 15 with MHE. Values significantly different from controls are indicated by asterisks. $^{**}P < 0.01$; $^{***}P < 0.001$.

When patients were classified as good or bad drivers according to the results in the simulator, only 3 (8.3%) of 36 patients without MHE were classified as bad drivers. In contrast, 8 (53%) of 15 patients with MHE were classified as bad drivers. This means that 40 of 51 (78.4%) patients were good drivers and 11 (21.6%) were bad drivers. 73% of bad drivers have MHE and only 17% of good drivers have MHE.

Driving ability was also impaired in patients with MHE when assessed using the Driver Test Battery. None of the parameters analysed was affected in patients without MHE, except bimanual coordination, which was slightly impaired already in patients without MHE. In contrast, all parameters analysed were altered in patients with MHE (Table 2).

For the speed of anticipation, both MDT and MDD were increased (by 78–79%; $P = 0.001$) in patients with MHE.

Bimanual coordination was strongly altered in patients with MHE, the total number of errors (TN)

Table 2. Values of the different parameters of SIMUVEG and driver test in the different groups of cirrhotic patients and in controls

Test	Parameter	Controls (n = 22)	Patients without MHE (n = 36) P vs. control	Patients with MHE (n = 15) P vs. control	% control*	Patients with MHE P vs. without EHM	Global ANOVA P values
SIMUVEG	MINTL	17 ± 1	20 ± 1	27 ± 4 P = 0.003	159	P = 0.001	0.003
	Speed	80 ± 2	77 ± 1	60 ± 2 P < 0.001	75	P < 0.001	<0.0001
Anticipation of speed	MDT	0.4 ± 0.05	0.4 ± 0.03	0.71 ± 0.07 P = 0.001	178	P = 0.001	<0.0001
	MDD	28 ± 3	31 ± 2.5	51 ± 5 P < 0.001	179	P = 0.001	<0.0001
Bimanual coordination	TT	3.9 ± 0.8	7.2 ± 1.1	37 ± 5 P < 0.001	943	P < 0.001	<0.0001
	TN	21 ± 4	31 ± 4	72 ± 3 P < 0.001	179	P < 0.001	<0.0001
	EP	2.0 ± 0.5	3.9 ± 0.6	20 ± 3 P < 0.001	985	P < 0.001	<0.0001
Multiple discriminative reactions	MRT-3	0.86 ± 0.04	0.99 ± 0.04	1.2 ± 0.05 P = 0.027	140	ns	0.025
	RA-3	33 ± 0.7	31 ± 0.7	28 ± 1.9 P = 0.041	84	ns	0.041
	ER-3	2.6 ± 0.6	4.7 ± 0.7	5.0 ± 1.4	192	ns	ns
	NA-3	0.11 ± 0.1	0.27 ± 0.1	2.9 ± 1.3 P = 0.002	2618	P = 0.001	0.001
Concentrated attention and resistance to monotony	MRT-4	0.58 ± 0.04	0.57 ± 0.02	0.81 ± 0.06 P = 0.016	140	P = 0.002	<0.0001
	RA-4	59 ± 0.35	59 ± 0.4	51 ± 3.7 P = 0.011	86	P = 0.006	0.005
	ER-4	1.2 ± 0.3	1.4 ± 0.3	8.5 ± 3.4 P = 0.006	607	P = 0.003	0.002
	NA-4	0.05 ± 0.05	0.24 ± 0.09	0.58 ± 0.39	1160	ns	ns

*% of the mean of patients with MHE with respect to the control mean.

Values are expressed as the mean ± SEM. The number of individuals for each parameter is indicated in parenthesis.

MINTL, percentage of the length of the circuit that a person has been driving incorrectly; MDT, mean deviation time in seconds; MDD, mean deviation distance in pixels; TT, total time of error with both hands in seconds; TN, total number of errors with both hands; EP, error percentage over the total trajectory; MRT-3, MRT-4, mean reaction time of right and wrong answers in seconds; RA-3, RA-4: number of right answers; ER-3, ER-4, number of errors; NA-3, NA-4, number of not answered stimuli; ns, difference no significant.

and the total time of errors with both hands (TT) were increased about 10-fold compared with control subjects (Fig 2A and B).

In the test of multiple discriminative reactions, the MRT of the answers (MRT-3) was increased in patients with MHE, indicating a slowing in the responses (Fig. 2C). The main effect of MHE was a very strong increase in the number of not answered responses (NA-3), which increased by 26-fold compared with control subjects. This parameter is not significantly affected in patients without MHE.

Patients with MHE performed worse than patients without MHE in concentrated attention and resistance to monotony tests. Patients with MHE showed a strong reduction (P = 0.006) in the number of right answers (RA-4) and again a slowing in the responses (Fig. 2D), with increased mean reaction time to answer (MRT-4) (P = 0.002) and a strong increase (six-fold) in the number of errors (ER-4).

Performance in psychometric tests of good and bad drivers

Table 3 shows the performance in the different psychometric tests of patients classified as good or bad drivers as above. Bad drivers (patients who do not keep driving abilities) showed worse performance compared with good drivers in the PHES and in all its individual tests except in the digit symbol test (Table 3). Performance in bimanual coordination and concentrated attention and resistance to monotony tests was especially affected (Table 3). Performance in multiple discriminative reactions was also reduced in bad drivers while anticipation of speed was not affected (Table 3).

Alterations in the NO-cGMP system, nitrotyrosine, ammonia and inflammatory parameters

Plasma cGMP levels were higher (P = 0.001) in bad drivers (24 ± 4 nM) than in good drivers (11 ± 1 nM)

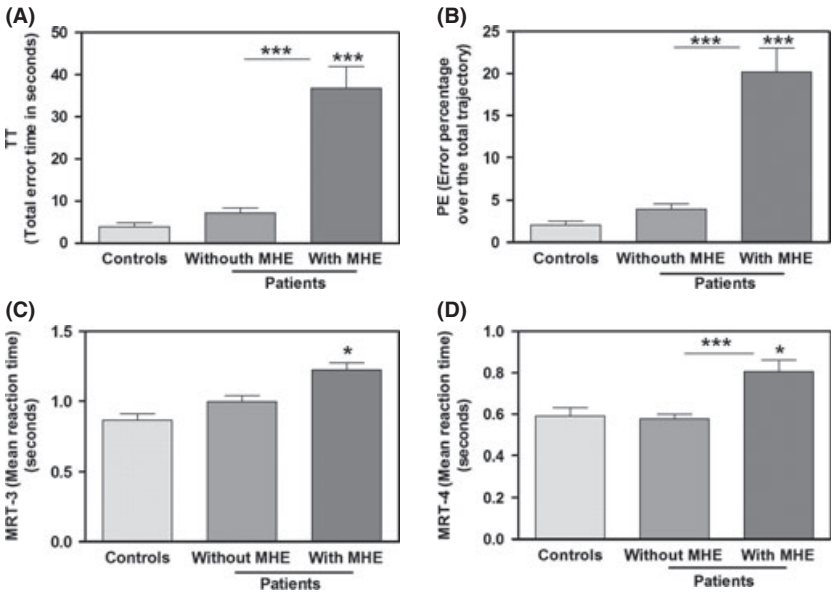

Fig. 2. Patients with minimal hepatic encephalopathy (MHE) show strong impairment of bimanual coordination and slowed reaction times. The results of the bimanual coordination tests are shown in (A) TT, total time of error with both hands and (B) EP, error percentage over the total trajectory. The mean reaction times (MRT-3 and MRT-4) of right and wrong answers in the Multiple discriminative reactions tests (C) and in the Concentrated attention and resistance to monotony tests (D) are also shown. Values are the mean ± SEM of 22 controls, 36 patients without and 15 with MHE. Values significantly different from controls are indicated by asterisks. *$P < 0.05$; ***$P < 0.001$.

Table 3. Performance in psychometric tests and driver test battery in patients with liver disease classified according to their driving ability in the driving simulator

Test	Parameter	Good drivers	Bad drivers	Bad drivers P vs. good drivers
PHES		−1.3 ± 0.5	−5 ± 1	0.001
Digit symbol test		−0.28 ± 0.11	−0.18 ± 0.26	ns
Number connection test A		−0.20 ± 0.11	−1.0 ± 0.36	0.007
Number connection test B		−0.28 ± 0.14	−1.64 ± 0.39	<0.001
Serial dotting test		−0.45 ± 0.14	−1.09 ± 0.34	0.05
Line tracing test		−0.08 ± 0.13	−0.82 ± 0.3	0.012
CFF (Hz)		41 ± 0.4	39.6 ± 0.4	ns (0.091)
Anticipation of speed	MDT	0.52 ± 0.04	0.55 ± 0.08	ns
	MDD	37 ± 3	39 ± 6	ns
Bimanual coordination	TT	11 ± 2	33 ± 8	<0.001
	TN	39 ± 4	59 ± 7	0.022
	EP	6 ± 1	18 ± 4	<0.001
Multiple discriminative reactions	MRT-3	1.02 ± 0.04	1.10 ± 0.08	ns
	RA-3	31 ± 1	26 ± 2	0.018
	ER-3	4.3 ± 0.7	7 ± 1	ns
	NA-3	0.55 ± 0.22	2.4 ± 1.7	0.047
Concentrated attention and resistance to monotony	MRT-4	0.61 ± 0.02	0.76 ± 0.09	0.036
	RA-4	57 ± 1	51 ± 5	0.04
	ER-4	2.4 ± 0.8	7.7 ± 4.4	0.05
	NA-4	0.22 ± 0.09	0.88 ± 0.58	0.046

Values are expressed as the mean ± SEM. Differences between groups were analysed using univariate analysis of covariance (ANCOVA) with age included as covariate, followed by post-hoc Bonferroni.

PHES, Psychometric Hepatic Encephalopathy Score; CFF, Critical Flicker Frequency; MDT, mean deviation time in seconds; MDD, mean deviation distance in pixels; TT, total time of error with both hands in seconds; TN, total number of errors with both hands; EP, error percentage over the total trajectory; MRT-3, MRT-4, mean reaction time of right and wrong answers in seconds; RA-3, RA-4: number of right answers; ER-3, ER-4, number of errors; NA-3, NA-4, number of not answered stimuli; ns, difference no significant.

Table 4. Biochemical parameters in patients with liver disease classified according to their driving ability in the driving simulator

Parameter	Good drivers	Bad drivers	Bad drivers P vs. good drivers
Nitrates + Nitrites (μM)	27 ± 2	24 ± 4	ns
cGMP in plasma (pmol/ml)	11 ± 1	21 ± 4	0.001
Basal cGMP in lymphocytes (pmol/mg prot)	0.08 ± 0.01	0.05 ± 0.01	ns
SNAP-induced cGMP increase (fold)	26 ± 2	38 ± 4	0.028
IL-6 (pg/ml)	7 ± 0.7	12 ± 1.0	0.003
IL-18 (pg/ml)	26 ± 4	74 ± 14	<0.001
NO-Tyr (nM)	11 ± 3	47 ± 13	<0.001
Ammonia (μM)	150 ± 3	146 ± 6	ns

Values are expressed as the mean ± SEM. Differences between groups were analysed using univariate analysis of covariance (ANCOVA) with age included as covariate, followed by post-hoc Bonferroni. The number of individuals for each parameter is indicated in parenthesis.

cGMP, cyclic guanosine monophosphate; IL-6, IL-18, Interleukin-6, Interleukin-18; SNAP, S-nitroso acetyl penicillamine; NO-Tyr, 3-Nitrotyrosine; ns, difference no significant.

(Table 4). NO-induced activation of soluble guanylate cyclase in lymphocytes was also higher ($P = 0.028$) in bad drivers (38 ± 4-fold) than in good drivers (26 ± 2-fold) (Table 4).

Intracellular cGMP in lymphocytes was strongly reduced in patients compared with controls ($P < 0.0001$), but was not different between good and bad drivers (Table 4). A similar effect occurs for the NO metabolites (nitrites+nitrates) and for ammonia levels, which were significantly higher in patients than in controls, but were not different in good and bad drivers (Table 4).

The inflammatory cytokines IL-6 and IL-18 were also significantly increased in bad drivers. IL-6 was higher ($P = 0.003$) in bad drivers (12 ± 1 pg/ml) than in good drivers (7 ± 0.7 pg/ml). Bad drivers showed 74 ± 14 pg/ml IL-18, which was higher ($P < 0.001$) than in good drivers (26 ± 4 pg/ml) (Table 4).

3-nitrotyrosine was increased 4.3-fold ($P < 0.001$) in bad drivers (47 ± 13 nM) compared with good drivers (11 ± 3 nM) (Table 4).

Logistic regression analysis to assess which functional alterations or biochemical parameters contribute to impairment of driving ability

On univariate analysis, altered driving ability was significantly associated with the presence of MHE, with the subtests NCT-A, NCT-B and LTT from PHES, with TT and EP from bimanual coordination test and RA-3 from multiple discriminative reactions tests (Table 5).

Bad performance in driving was also significantly associated with plasma cGMP, SNAP-induced cGMP increase in lymphocytes, IL-6, IL-18 and 3-nitrotyrosine levels (Table 5).

Table 5. Univariate and multivariate logistic regression analyses to predict driving ability

	OR	95% CI	*P*
Univariate logistic regression analyses			
Independent variables			
MHE (+)	12.57	2.648–59.677	0.002
Number connection test A	0.416	0.201–0.861	0.018
Number connection test B	0.350	0.180–0.681	0.002
Line tracing test	0.407	0.188–0.880	0.022
TT	1.072	1.019–1.127	0.007
EP	1.135	1.035–1.245	0.007
RA-3	0.817	0.678–0.984	0.033
NA-3	1.329	0.933–1.893	0.115
RA-4	0.924	0.841–1.016	0.102
cGMP in plasma	1.134	1.035–1.242	0.007
SNAP-induced cGMP increase (fold)	1.047	1.003–1.094	0.038
3-Nitro-tyrosine	1.035	1.011–1.060	0.004
IL-6	1.255	1.055–1.494	0.011
IL-18	1.038	1.015–1.061	0.001
Multivariate logistic regression analysis			
Predictor variables			
NO-Tyr	1.037	1.005–1.070	0.021

On both uni- and multivariate analysis, the dependent variable was driving ability, according to parameters of Driving Simulator. On multivariate analysis, independent variables were those that were significant ($P < 0.05$) on univariate analysis.

MHE (+), with MHE; Number connection test A, Number connection test B and Line tracing test are subtests from PHES battery; Parameters from Bimanual Coordination test: TT, total time of error with both hands in seconds; EP, error percentage over the total trajectory. Parameters from Multiple discriminative reactions test: RA-3, number of right answers; NA-3, number of not answered stimuli. Parameters from Concentrated attention and resistance to monotony test: RA-4, number of right answers. cGMP, cyclic guanosine monophosphate; IL-6, IL-18, Interleukin-6, Interleukin-18; SNAP, S-nitroso acetyl penicillamine; NO-Tyr: 3-Nitrotyrosine. OR, Odds ratio; CI, Confidence Interval.

Performance in the PHES, IL-6 and IL-18 correlated with performance in bimanual coordination tests (Fig. 3).

Multivariate logistic regression analysis, using as dependent variable driving performance and as independent variables psychometric tests and biochemical parameters significantly different on univariate analyses, showed that only 3-nitrotyrosine concentration was significantly associated with bad driving (OR: 1.037; 95% CI: 1.05–1.07; $P = 0.021$) (Table 5).

Discussion

The results reported show that patients with MHE have impaired driving ability with reduced driving speed and impaired lateral control of the vehicle, which correlates with the grade of MHE.

A remarkable effect of MHE was that lateral control of the vehicle was reduced in spite of reduced driving speed. In control subjects and in patients without MHE,

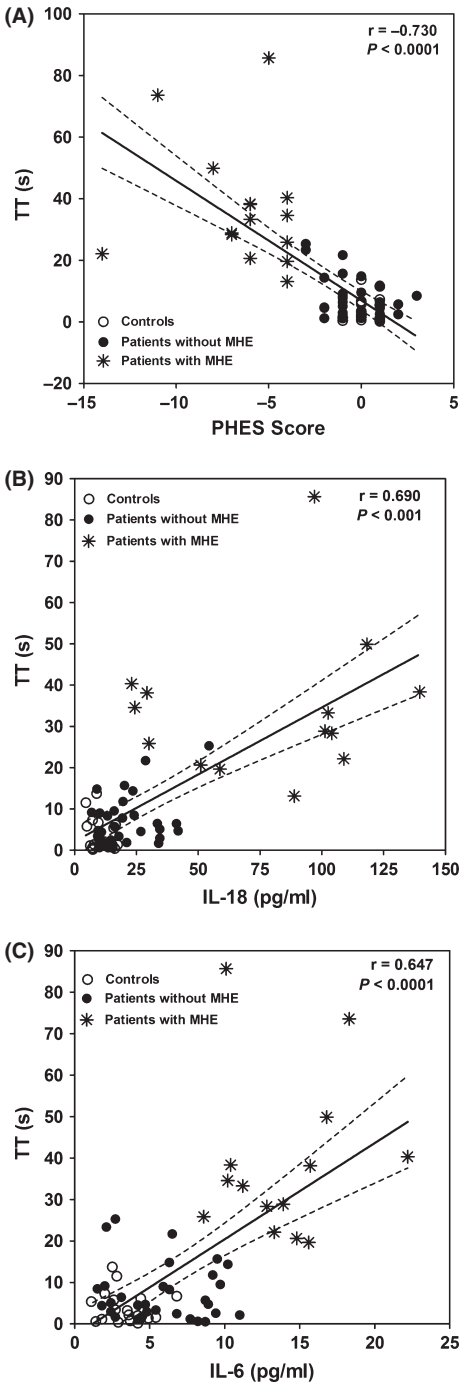

Fig. 3. Alterations in bimanual coordination correlate very well with the grade of minimal hepatic encephalopathy (MHE) (performance in the PHES) and with serum levels of pro-inflammatory cytokines IL-18 and IL-6. The correlations between total time of error with both hands (TT) in the bimanual coordination tests with performance in the PHES (A), serum levels of IL-18 (B) and of IL-6 (C) are shown.

there was a positive correlation between low lateral control of the vehicle (high MINTL) and speed ($r = 0.707$, $P = 0.0007$ and $r = 0.607$, $P = 0.0008$ for controls and patients without MHE respectively). This indicates that, as expected, lateral control was lower when speed was higher (Fig. 1E). Usually, drivers choose a speed that allows for a comfortable lateral control, and consequently drivers with limited control abilities reduce speed to maintain reasonable lateral control. However, for patients with MHE, there was a negative correlation ($r = -0.682$, $P = 0.005$), indicating that MHE patients showed very low lateral control despite driving at a low speed (Fig. 1E), suggesting a severe impairment of driving ability.

Kircheis *et al.* (8) reported that patients with MHE showed driving deficits both in real on-road driving and in psychometric tests and suggested that, for assessment of driving ability, real or simulated driving tests are required (8). The validity of driving simulators to evaluate driving deficits has been clearly established. Driving simulators overcome methodological constraints of real-world evaluation of driving performance and predict real-world outcomes, including accident reports 5 years later (34–36). Simulators are especially valid for analysis of speed and lateral position (37), the two parameters assessed in our study.

Patients with MHE show impaired driving performance both in on-road driving (7, 8) and in driving simulators (10). Taking into account the above studies, we classified the patients as good or bad drivers according to performance in the driving simulator. This classification showed that 92% of patients without MHE were good drivers while 53% of patients with MHE showed impaired driving ability. We found that CFF show a weak association while PHES was strongly related to fitness to drive. CFF and PHES have been considered complementary in the assessment of MHE (28, 29).

Although both are useful to detect the presence of MHE, the two procedures evaluate different cerebral processes. CFF may reflect damage in various cortical areas even though flicker is initially processed in the occipital cortex. CFF is useful to assess alterations in visual signal processing and for detection of arousal or attention abnormalities, but not for alterations in motor function (38). PHES explores visual perception, construction and visual/spatial orientation together with motor speed, accuracy, concentration and attention.

The weak association of CFF with fitness to drive suggests that alterations in the processes evaluated by CFF are not the main contributors to impairment of driving ability, but may contribute to other neuropsychological abnormalities in MHE. In contrast, parameters evaluated by PHES (e.g. motor coordination, fine motor skills) are the main contributors to driving impairment. Our results showing that PHES was strongly related to fitness to drive agree with several studies showing an association of MHE (diagnosed with paper and pencil tests) and driving impairment (7, 10–12).

Performance in the psychometric tests showed that patients with MHE had a strong impairment of

bimanual coordination, reduced visuo-motor coordination, slowed speed of anticipation, impaired concentrated attention and needed more time to react to different situations in multiple discriminative reactions and concentrated attention and resistance to monotony tests. Patients with MHE also showed higher tendency towards impulsivity, impaired ability to safely adapt to traffic situations requiring time and space estimations (e.g. an overtaking), impaired skills and precision in connecting visual information and actions (e.g. changing gear) and poor resistance to monotony, indicative of higher fatigue potential.

The analyses of the data suggest that psychomotor slowing and longer reaction times contribute to impairment of driving ability in patients with MHE. Psychomotor slowing would also contribute to the large increase in not answered responses in the tests of multiple discriminative reactions and of concentrated attention and resistance to monotony. Patients with MHE did not have enough time to react and did not provide a response. This would be very dangerous in real driving situations, increasing the risk of accidents.

A possible limitation of this study is that the age of the group with MHE was slightly higher (although not statistically different) than in the other groups. This could alter the driving skills and/or their performance using computers for the driver tests. However, performance of patients with MHE in some computer tests was not different from controls or patients without MHE, while performance in other tests was impaired. Moreover, all comparisons between groups were performed including age as a covariate. This suggests that impaired driving and performance in psychometric tests was owing to MHE and not to age-related biases.

Anstey *et al.* (39) proposed that reaction time, information-processing speed, visual attention, short-term memory and executive function are all associated with safe driving in older adults. The results reported here support that impairment of these parameters is a main contributor to reduced driving performance in MHE. The association of MHE and driving impairment shown here agrees with previous studies (7, 8, 12, 40). Our results support that impairment of mental processing speed, of attention (the functions evaluated by NCT-A and NCT-B) and of motor coordination are main contributors to reduced driving performance in patients with MHE. This is in agreement with a report from Bajaj *et al.* (11) that showed also that cirrhotic patients with MHE have a significantly higher crash rate than patients without MHE.

Patients with MHE also showed impaired performance under repetitive, monotonous driving conditions, evaluated by the concentrated attention and resistance to monotony test. This is in agreement with the higher rate of driving-associated fatigue, predictive of simulator collisions, reported by Bajaj *et al.* (41).

Patients with MHE showed a wide array of neurological alterations including reduced attention, mental processing speed and visuo-motor coordination and psychomotor slowing, which contribute to impair driving ability. Impairment in these cerebral processes would be caused by different pathogenic mechanisms to which hyperammonaemia, inflammation and other alterations such as oxidative stress may contribute differentially.

The main contributors to the cognitive and motor alterations in HE are hyperammonaemia and inflammation (18–22). The results reported here show that hyperammonemia is not a good predictor of driving impairment. This suggests that hyperammonaemia would not be enough to induce driving impairment in patients with MHE, but would be necessary to act synergistically with other alterations (mainly inflammation) to impair their driving performance.

The pro-inflammatory cytokines IL-6 and IL-18 are associated with bad driving, supporting that inflammation would contribute to alterations involved in impairment of fitness to drive.

Increased nitrotyrosine levels are associated with poor driving performance. Nitrotyrosine has been proposed as a marker of oxidative stress (42) and of the presence of MHE in patients with cirrhosis (24). The present results suggest that inflammation and oxidative stress would be relevant contributors to the cerebral alterations that finally lead to impaired fitness to drive in patients with MHE.

This study confirms the poor driving performance of patients with MHE and shed light on some of the neurological alterations contributing to this impairment. It would be important to make efforts to prevent impaired driving. In daily clinical practice, diagnosis and treatment of MHE in cirrhotic patients using cheap, wide-distributed and available methods such as the PHES and determination of serum nitrotyrosine could save lives and society money by reducing motor vehicle accidents among MHE patients.

In conclusion, impairment of mental processing speed, attention and alterations in visuo-spatial and motor coordination seem main contributors to impaired driving ability in patients with MHE. Increased serum nitrotyrosine levels are associated with impaired driving in patients with MHE.

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