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Asymmetric Di-methyl Arginine is Strongly Associated with Cognitive Dysfunction and Brain MR Spectroscopic Abnormalities in Cirrhosis

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Abstract

Background—Asymmetric di-methyl arginine (ADMA) is an inhibitor of nitric oxide synthase that accumulates in liver disease and may contribute to hepatic encephalopathy(HE).

Aim—To evaluate the association of ADMA with cognition and brain MR spectroscopy(MRS) in cirrhosis.

Methods—Cirrhotic patients with/without prior HE and non-cirrhotic controls underwent cognitive testing and ADMA determination. A subgroup underwent brain MRS [Glutamine/ glutamate(Glx), myoinositol(mI), N-acetyl-aspartate(NAA) in parietal white, occipital gray and anterior cingulate(ACC)]. We also tested cognition and ADMA in a cirrhotic subgroup before and 1 month after transjugular intrahepatic portosystemic shunting (TIPS). Cognition and MRS values were correlated with ADMA and compared between groups using multi-variable regression. ADMA levels were compared between those who did/did not develop post-TIPS HE.

Results—90 cirrhotics (MELD13, 54 prior HE) and 16 controls were included. Controls had better cognition and lower ADMA, Glx and higher mI compared to cirrhotics. Prior HE patients had worse cognition, higher ADMA and Glx and lower mI compared to non-HE cirrhotics. ADMA was positively correlated with MELD ($r=0.58, p<0.0001$), abnormal cognitive test number($r=0.66, p<0.0001$) and Glx and NAAA (white matter,ACC) and negatively with mI. On regression, ADMA predicted number of abnormal tests and mean Z-score independent of prior HE

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and MELD. 12 patients underwent TIPS; 7 developed HE post-TIPS. ADMA increased post-TIPS in patients who developed HE ($p=0.019$) but not in others ($p=0.89$).

Conclusions—A strong association of ADMA with cognition and prior HE was found independent of MELD score in cirrhosis.

Keywords

hepatic encephalopathy; TIPS; ADMA; pathogenesis

Introduction

Asymmetric di-methyl arginine (ADMA) is a by-product of amino acid metabolism that can interfere with nitric oxide synthesis by competitive inhibition of endothelial nitric oxide synthase (eNOS)[11]. Increased levels of ADMA are associated with endothelial dysfunction. ADMA may contribute to brain dysfunction in patients with Alzheimer's disease and stroke[19]. ADMA is degraded by di-methylarginine di-methylaminohydrolase (DDAH) which is localized primarily in the liver and kidney. ADMA levels increase with worsening severity of liver disease in cirrhotic patients and animal models[12, 17, 23]. Hepatic encephalopathy (HE) is a major complication of patients with cirrhosis, in which toxic effects of ammonia on cerebral astrocytes is thought to play a key pathogenetic role[2, 10, 20]. In rodent studies, the ADMA pathway in the brain has been responsive to changes in ammonia[3]. However the role of ADMA as a pathogenic mechanism or a target for therapeutic intervention in human HE is not clear.

The aim of this study was to evaluate the association of ADMA with cognitive function and brain MR spectroscopy in patients with cirrhosis in a cross-sectional and longitudinal manner. The *a priori* hypothesis was that ADMA was associated with cognitive function and brain MRS independent of the liver disease severity.

Patients and Methods

This was a study with cross-sectional and prospective components.

Cross-sectional study

Patients with cirrhosis and healthy, age-matched controls were included prospectively after written, informed consent. Cirrhosis was diagnosed based on liver biopsy, radiological appearance or endoscopic appearance of varices in patients with chronic liver disease. Subjects who were actively abusing alcohol or illicit drugs, those with focal neurological deficits and those unable to give consent were excluded. A detailed history of complications of cirrhosis including HE and HE therapy with lactulose and/or rifaximin was recorded. All patients underwent a detailed neuro-psychiatric examination with mini-mental status exam and a standard psychometric battery consisting of the psychometric hepatic encephalopathy score (PHES), block design test (BDT) and inhibitory control tests (ICT)]. Blood was drawn for MELD score, venous ammonia and a portion of the serum was stored at -80°C . The serum was subsequently analyzed for ADMA, innate immunity (IL-6, TNF- α), Th1 (IL-2, IL-1b), Th2 (IL-4, IL-10) and Th17 responses (IL-17 and IL-23), glial function [s100b protein], endothelial activation [soluble intravascular adhesion molecule (sICAM-1) and soluble vascular adhesion molecule (sVCAM-1)] and asymmetric di-methyl arginine (ADMA). These were analyzed in duplicate using published ELISA techniques by a standard reference laboratory, Assaygate Inc, Ijamsville, MD (mean assay coefficient of variation 4%; range from 0-18% for ADMA in our samples).

A subgroup of the patients and controls agreed to undergo MRS of the brain on the same day as the blood draw and cognitive testing. MRS testing was performed using the right parietal white matter (RPWM), occipital gray matter (OGM) and anterior cingulate cortex (ACC) as the regions of interest (ROI). The specific metabolites interrogated were the creatine ratios of choline, glutamate+glutamine (Glx), myoinositol (mI) and N-acetyl aspartate (NAA) as performed in prior HE studies[13].

Prospective study

A subgroup of these patients underwent TIPS procedure for elective purposes. The cognitive profile, venous ammonia and ADMA were repeated one month post-TIPS placement.

Statistical analysis

In the cross-sectional study, cognitive, laboratory measures including ADMA, inflammatory markers, endothelial activation markers and brain metabolite ratios were compared between controls and cirrhotics and then within cirrhotics with and without HE. A correlation analysis was performed between ADMA and cognitive performance, MELD score, venous ammonia and brain metabolite ratios for all three ROIs. Stepwise backward multi-variable regression was performed with cognitive performance (mean Z score and number of abnormal cognitive tests) as outcomes. The variables input were age, education status, venous ammonia, MELD score, diagnosis of HE and ADMA.

For the prospective study, the comparison of ADMA before and after TIPS was performed as a group at first and the change in ADMA in those who experienced an HE episode after TIPS was compared to those who did not develop HE post-TIPS.

This study was approved by the Institutional Review Boards at Virginia Commonwealth University and McGuire VA Medical Centers.

Results

Cross-sectional study

A total of 90 patients with cirrhosis and 16 age-matched healthy controls were included (Table 1). Controls had significantly better cognitive performance, lower ADMA, inflammatory markers and endothelial activation markers than cirrhotic patients (Table 1 and Figure 1). The majority of cirrhotic patients had HCV cirrhosis (n=45) followed by alcoholic (n=30) (of these 25 patients had both HCV and alcohol as the etiology) and other etiologies (n=15). Prior HE was observed in 54 (60%), of which there was relatively similar distribution of those on lactulose alone (n=29) compared to those on lactulose with rifaximin (n=25); all patients at the time of testing had a stage of HE grade 0 assessed by a normal MMSE. The mean MELD in the entire cirrhosis group was 13±9 and the venous ammonia levels were 51±29.

As was expected, patients with prior HE had a higher MELD, venous ammonia, IL-6, and endothelial activation markers (Table 2). ADMA levels in the cirrhosis group were significantly correlated with MELD score ($r=0.6$, $p<0.0001$, Figure 2) but not with age ($r=0.13$, $p=0.18$) or ammonia ($r=0.21$, $p=0.07$). There was also a significant correlation between MELD score and serum creatinine ($r=0.45$, $p=0.002$) and a negative correlation with serum albumin ($r=-0.56$, $p<0.001$). We found a significant correlation between ADMA and IL-6 (0.43, $p<0.0001$), IL-2 ($r=0.24$, $p=0.008$), IL-17 ($p=0.3$, $p=0.001$), s100b ($p=0.24$, $p=0.008$) and sICAM ($p=0.4$, $p<0.0001$). The rest of the markers tested were not significant. There was a significantly higher MELD and ADMA, and cognitive performance testing score (indicative of worse cognitive ability) in patients with both alcoholic and HCV as their

cirrhosis etiology compared to the other groups while other markers, including ammonia and inflammatory cytokines were not significantly different (Table 3).

Relationship of ADMA with cognition

There was a significant correlation between ADMA levels and NCT-A ($r=0.4$, $p<0.0001$), NCT-B (0.4 , $p<0.0001$), DST (-0.6 , $p<0.0001$), SDT (0.5 , $p<0.0001$), BDT (-0.4 , $p<0.0001$), LTT time (0.3 , $p=0.004$), Lures (0.5 , $p<0.0001$) and targets (-0.4 , $p=0.002$) and a trend with LTT errors (0.2 , $p=0.06$). MELD score had comparatively weaker correlations with NCT-A ($r=0.24$, $p=0.03$), NCT-B (0.32 , $p=0.005$), DST (-0.5 , $p<0.0001$), SDT (0.31 , $p=0.005$), BDT (-0.3 , $p=0.006$), lures (0.3 , $p=0.03$) but not with the line tracing test or targets. Therefore we found that ADMA was significantly correlated with impairments across a wide spread of domains; psychomotor speed (DST, SDT, NCT-A and B, ICT targets and lures), response inhibition (lures), visuo-motor coordination (BDT, LTT time) and sustained attention (targets). In the general population, norms were available for 6 test outcomes (NCT-A/B, DST, BDT, ICT lures and targets) and based on a two standard deviation cut-off from the norms, the number of abnormal tests of these six were calculated. Fifteen percent of cirrhotic patients were normal on all six, 18% were abnormal on one test, 22% on two test, 11% abnormal on three test, 8% abnormal on four tests, 21% abnormal on five tests and 4% were abnormal on all six test outcomes. As expected patients with prior HE had a significantly higher median number of abnormal tests (4 compared to 2, $p<0.0001$). The Z scores were calculated for each of these six test outcomes compared to the population norms and an average Z score was calculated. The average Z score were significantly worse in prior HE patients compared to those without prior HE (4.4 in prior HE compared to 1.5 without prior HE, $p<0.0001$). ADMA levels were significantly correlated with average Z score (Figure 3).

The stepwise backward regression analysis was performed with age, education, MELD score, ammonia, prior HE and ADMA with the (i) number of abnormal tests and (ii) average Z score for all tests using a stepwise regression analysis. Both ADMA and prior HE appeared as independent and the only significant predictors with either the average Z score [regression equation = $-0.84 + 2.04$ (prior HE) + 5.95 (ADMA), $R^2=28.0\%$, $p<0.0001$] or number of abnormal tests [regression equation = $0.49 + 1.10$ (prior HE) + 4.19 (ADMA), $R^2=41.7\%$, $p<0.0001$] as the outcomes; MELD did not add to the regression in both situations ($p=0.78$ for number of abnormal cognitive tests and $p=0.25$ for mean Z score). This shows that ADMA is associated with cognitive performance independent of MELD score.

Brain MR spectroscopy

A total of 46 subjects (6 controls and 40 cirrhotic patients) underwent MRS of the brain. Of the 40 cirrhotics, 20 had prior HE. The Glx ratios were significantly higher in cirrhotics compared to controls (RPWM, 2.4 ± 0.8 vs. 1.6 ± 0.24 , $p<0.0001$, OGM, 2.4 ± 0.8 vs. 1.9 ± 0.3 , $p=0.007$ and ACC, 2.7 ± 0.6 vs. 2.1 ± 0.3 , $p=0.008$). Conversely, the mI ratios were significantly lower in cirrhotics than controls (RPWM, 0.5 ± 0.4 vs. 0.9 ± 0.12 , $p=0.001$, OGM, 0.5 ± 0.2 vs. 0.7 ± 0.1 , $p=0.005$ and ACC, 0.5 ± 0.2 vs. 0.7 ± 0.1 , $p=0.02$). There were no significant differences between controls and cirrhotic patients on NAA and Choline ratios on any ROI. A significant negative correlation of ADMA was observed with mI ratios in all three ROIs (RPWM – 0.5 , $p=0.002$, OGM: -0.6 , $p<0.0001$ and ACC: -0.6 , $p=0.001$). There was a significant positive correlation of ADMA with Glx in the RPWM (0.6 , $p<0.001$) and ACC (0.4 , $p=0.02$) but not OGM (0.2 , $p=0.3$). Similar to Glx, the NAA ratios were positively correlated with ADMA in the RPWM (0.33 , $p=0.03$) and ACC (0.5 , $p=0.005$) but not the OGM (0.11 , $p=0.5$). No correlations were found between ADMA and choline levels. There was no significant difference in MRS values between patients with and without

alcoholic liver disease (RPWM Glx: 2.8 vs 2.2, $p=0.08$, mI: 0.5 vs. 0.6, $p=0.23$; OGM Glx 2.6 vs 2.3, $p=0.3$, mI: 0.5 vs 0.6, $p=0.3$; ACC Glx: 2.9 vs 2.6, $p=0.09$, mI: 0.54 vs 0.58, $p=0.8$). Mirroring this, we did not find any difference in MRS parameters in patients with and without hepatitis C (RPWM Glx: 2.5 vs 2.3, $p=0.7$, mI 0.5 vs 0.6, $p=0.5$; OGM Glx: 2.2 vs. 2.6, $p=0.08$, mI: 0.53 vs 0.63, $p=0.26$; ACC Glx: 2.7 vs 2.6, $p=0.9$, mI 0.5 vs 0.6, $p=0.12$).

Prospective study

A total of 12 patients were followed before and after insertion of an elective TIPS (seven for refractory ascites, four for hepatic hydrothorax and one for multiple variceal bleeding episodes). Patients were then followed at least for three months post-TIPS and development of overt HE post-TIPS was recorded. The development of overt HE post-TIPS was defined as evidence of disorientation and/or asterixis requiring therapy or hospitalization. Seven patients developed post-TIPS HE; all were spontaneous and had resolved with therapy before the ADMA levels were drawn. There was no significant change in the MELD score before and after TIPS (13 ± 6 pre vs 13 ± 5 post-TIPS). The mean pre-TIPS hepatic portal-venous pressure gradient was 14 ± 3 mmHg which reduced to 6 ± 2 mmHg after the procedure.

ADMA levels in the entire group did not change before and after TIPS (mean 0.45 pre and 0.48 post-TIPS, $p=0.61$) or in those who did not develop HE (mean 0.41 pre and 0.41 post, $p=0.91$). One particular patient who developed HE post-TIPS had a dramatic increase in his ADMA level from 0.43 to 1.04 after TIPS; he was excluded as an outlier and the remainder was analyzed; there was a significant increase in ADMA compared to baseline in those patients developing HE Post-TIPS (mean pre 0.44, post 0.53, $p=0.019$, Figures 4 and B).

Most of the cognitive tests in those who went on to develop HE after TIPS were significantly impaired at baseline compared to those who did not develop HE post-TIPS (Targets 97% vs 82%, $p=0.05$, NCT-B, 86 vs 185, $p=0.05$, Block design 27 vs 12, $p=0.007$, serial dotting 73 vs 108, $p=0.024$ but not line tracing time 112 vs 117, $p=0.8$, Lures 11 vs 16, $p=0.26$, NCT-A 48 vs 58, $p=0.5$ and Line tracing errors 29 vs 57, $p=0.08$). Interestingly, the pre-TIPS comparison of ADMA levels between those who did or did not develop HE post-TIPS was not significant (mean no-HE post-TIPS 0.41 vs. 0.44 in post-TIPS HE, $p=0.26$) but there was a significantly higher level of ADMA in post-TIPS patients who developed HE compared to those who remained HE-free (median post-TIPS HE 0.53 vs post-TIPS no HE, 0.41, $p=0.011$).

Discussion

Patients with cirrhosis had a significantly higher ADMA compared to healthy controls, which was significantly correlated with worse cognitive function and with high Glx and low mI on brain MRS. The prospective analysis after TIPS placement showed that ADMA levels in patients who developed HE post-TIPS were significantly higher in those who developed HE compared to those who did not although the pre-TIPS levels were similar.

ADMA is considered to be an end-product of nitric oxide that can result in reverse inhibition and a false-transmitter[11]. While this role can associate ADMA with endothelial dysfunction and poor cardiovascular outcomes in patients without liver disease, its role in cirrhosis is complicated by the localization of DDAH in the liver[17, 18]. We replicated prior studies in which we found a correlation between ADMA and MELD score and ADMA levels were significantly higher in patients with HE and we demonstrated its correlation with markers of Th1 and Th17 immune response[12, 23]. There is evidence linking ADMA and nitric oxide regulation with cognitive dysfunction and oxidative stress[7]. The resultant reduction in cerebral blood flow has been shown in healthy controls and patients with neuro-

degenerative disorders[9]. A recent animal study also found increased brain ADMA in BDL rats with reduction in eNOS and DDAH brain activity that recovered after therapy with ornithine phenylacetate, a compound that reduces ammonia and potentially inflammation[3]. Interestingly gene therapy using DDAH in this model reduced the portal pressure further extending these findings[14].

Cognitive deficits in HE and chronic liver disease are intimately linked to changes in cerebral blood flow[5, 26]. Therefore not surprisingly, we found a significant correlation between cognitive dysfunction and ADMA, which was stronger than the correlation between cognitive dysfunction and MELD score. This spanned several domains ranging from spatial function to response inhibition[2]. These findings are interesting because a recent study on developing rats showed that in the BDL model, with and without hyperammonemia, ADMA levels increased and were correlated with tests of spatial cognition[8]. This was especially significant because there was an increase in both cerebral and peripheral ADMA in the development of this spatial cognitive dysfunction. These cognitive tests are similar to what were tested and found to be abnormal in patients with HE[24]. Nitric oxide production is not only important for cerebral blood flow and cognition, but it can also quench oxidative stress via formation of peroxynitrite that undergoes dismutation to 3-nitrotyrosine[1]. Interestingly 3-nitrotyrosine levels are higher in MHE independent of ammonia levels[15]. It is also possible that high ADMA levels, like shown in prior animal studies, are likely to uncouple eNOS, leading to superoxide generation and oxidant injury and may be an additional mechanism behind the insults after TIPS insertion [3, 12]. This leads us to speculate that the inflammatory-oxidative stress axis may be a complementary and important pathway of HE pathogenesis.

We also found a direct relationship between ADMA and changes in brain MRS typical for HE[13]. Specifically with the increase in intra-cerebral ammonia, the accumulation of glutamate/glutamine and the compensatory reduction of myo-inositol is expected[6]. ADMA was correlated with increasing Glx and reduced mI in all three brain regions tested. While this can only prove correlation, studies have shown that in BDL cirrhotic rats, ADMA increases along with ammonia in the cerebral tissue[3]. Interestingly, this correlation with increased Glx and reduced mI was found in all three ROIs indicating the non-focal nature of this cerebral metabolic defect. Since ADMA has been related to intra-cerebral vascular regulation, we can speculate that ADMA levels could be involved in the alteration in their brain blood flow related to HE which is associated with cognitive dysfunction[5, 22].

To study the relationship prospectively, we studied a homogeneous group of patients before and after TIPS placement. Similar to prior published experience, there was no significant change in ADMA levels after TIPS placement in the entire group[21]. We also did not find a difference in the baseline ADMA levels between those who did or did not develop HE ultimately. However post-TIPS ADMA levels in those who developed HE were significantly higher compared to their baseline as well as to those who did not develop post-TIPS HE, which is a novel finding. This indicates that ADMA levels change with new development of HE after TIPS. Since all post-TIPS HE patients had already developed HE before retesting for ADMA it is difficult to evaluate whether ADMA elevation occurs before or after the HE episode. However, merely the insertion of TIPS did not change ADMA levels significantly but development of HE did, in the presence of a statistically similar MELD score pre and post-TIPS. This supports the notion that the development of HE post-TIPS is related to peripheral ADMA levels in the prospective study, although the exact mechanism is not clear.

Since we were able to find worse cognition, worse brain MRS parameters and increased ADMA and key inflammatory markers in prior HE patients despite normal mentation (grade

0 at testing) and adequate therapy with lactulose and rifaximin in our population, it is probable that therapies targeting additional dimensions of HE patho-physiology need to be studied[20]. The treatment options needed could potentially target inflammation and ADMA production in the brain, thereby increasing our control of these cognitive issues in HE[4]. It could be speculated that the use of ornithine phenylacetate, which has been shown to reduce brain ADMA along with ammonia by supplementing DDAH in animals could be such an option but human studies are required[3, 16, 25].

Therefore we conclude that ADMA is associated with cognitive dysfunction and inflammation in cirrhosis independent of the severity of liver disease. We also found that ADMA levels are correlated with brain metabolite ratios suggesting high Glx and low mI in several regions on MR spectroscopy. ADMA levels were also significantly higher in those who developed HE after TIPS placement compared to those who remained free of HE. Our results indicate the linkage of ADMA with cognitive function in cirrhotics and the need to study therapies directed at ameliorating the oxidative stress it represents in the cirrhotic population.

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Abbreviations

ADMA	asymmetric dimethyl arginine
HE	hepatic encephalopathy
eNOS	endothelial nitric oxide synthase
DDAH	di-methylarginine di-methylaminohydrolase
BDL	bile-duct ligated
MRS	magnetic resonance spectroscopy
TIPS	transjugular intra-hepatic porto-systemic shunting
sICAM-1	soluble intravascular adhesion molecule
sVCAM-1	soluble vascular adhesion molecule
PHEs	psychometric hepatic encephalopathy score
ICT	inhibitory control test
BDT	block design test
Glx	Glutamine/glutamate
mI	myoinositol
NAA	N-acetyl-aspartate
ACC	anterior cingulate cortex

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Figure 1. ADMA concentrations between controls, cirrhotic patients with and without prior HE
The mean ADMA levels are significantly different between the groups, with the lowest levels in controls and the highest in cirrhotic patients with prior HE ($p < 0.0001$). Individual values are represented by open circles while the black circle and bars indicate the mean and 95% confidence intervals for the mean of the ADMA concentrations.

Figure 2. Correlation of ADMA with MELD score in cirrhotic patients with and without prior HE

There was a significant positive correlation between MELD score and ADMA in the cirrhosis group with the higher MELD and ADMA occurring in the prior overt HE (OHE) group indicated by black squares compared to the lower MELD and ADMA in the cirrhotic group without overt HE indicated by open circles.

Figure 3. Correlation between ADMA and cognitive performance

ADMA levels in the cirrhosis group were positively correlated with number of abnormal tests (ranging from 0 to 6). The cirrhotic group with prior overt HE (OHE), indicated by the black squares, was significantly more cognitively impaired compared to the group without overt HE represented by the open circles.

Figure 4. ADMA levels in patients who did and did not develop HE after TIPS placement
ADMA levels (in μM) rose significantly from their pre-TIPS baseline in each of the seven patients who developed post-TIPS HE (one was excluded since his levels increased by 0.61 μM to exclude undue influence by an outlier) (Figure 4A) but no significant trend was seen in the remaining 5 patients who did not develop post-TIPS HE (Figure 4B). The median of the differences was also significant between groups.

Table 1

Comparison between cirrhotic patients and healthy controls

	Cirrhosis (n=90)	Controls (n=16)	P value
Age (years)	54±8	52±7	0.31
Education (years)	14±1	15±3	0.21
Cognitive testing			
Higher score is worse			
Number connection A (sec)	45±26	24±6	<0.0001
Number connection B (sec)	127±98	54±15	<0.0001
Line tracing time (sec)	104±39	78±22	0.001
Line tracing errors (number)	37±31	19±15	0.001
Serial dotting (sec)	80±30	49±12	<0.0001
ICT lures (number)	15±9	4±2	<0.0001
Lower score is worse			
Digit Symbol (raw score)	48±17	76±10	<0.0001
Block Design (raw score)	30±30	49±12	<0.0001
ICT targets (% correct)	91±12	99±1	<0.0001
ADMA (μM)	0.46±0.17	0.28±0.05	<0.0001
IL-1b (pg/ml)	10.8±38.8	0.68±2.06	0.01
IL-2(pg/ml)	14.9±50.6	0.39±1.55	0.005
TNF-alpha (pg/ml)	21.4±43.4	0.0±0.0	<0.0001
IL-6 (pg/ml)	30.0±52.4	11.9±33.8	<0.0001
IL-17 (pg/ml)	11.2±38.7	0.23±0.91	0.005
IL-23 (pg/ml)	1268±3182	239±849	0.008
IL-4 (pg/ml)	23.1±85.5	0.33±1.34	0.008
IL-10 (pg/ml)	7.7±20.8	0.61±1.17	0.001
s100b (pg/ml)	52.4±97.4	20.2±16.1	0.003
sICAM (μg/ml)	0.29±0.22	0.12±0.28	<0.0001
sVCAM (μg/ml)	0.18±0.10	0.78±0.16	<0.0001

ICT: inhibitory control test, sVCAM: soluble vascular adhesion molecule, sICAM: soluble inter-cellular adhesion molecule

Table 2

Comparison between patients with and without prior HE

	Cirrhotics with prior HE (n=46)	Cirrhotics with prior HE (n=54)
Age	56±5	57±6
Education (years)	14±2	13±4
Venous Ammonia (μmol/l)	42.6±29.8	56.4±26.2 *
MELD score	9.84±3.54	16.13±6.35 *
ADMA (μM)	0.38±0.10	0.54±0.2 *
IL-2 (pg/ml)	10.0±45.4	19.6±55.6
IL-1b (pg/ml)	15.0±56.3	7.3±12.0
TNF-alpha(pg/ml)	12.5±33.6	7.56±7.16
IL-6 (pg/ml)	11.9±33.8	30.0±52.4 *
IL-17 (pg/ml)	9.3±24.3	13.2±48.3
IL-23 (pg/ml)	1035±2842	1427±3484
IL-4 (pg/ml)	23.3±95.1	23.6±78.9
IL-10 (pg/ml)	9.1±29.5	6.47±8.84
s100b (pg/ml)	33.0±28.8	68±129
sICAM (μg/ml)	0.29±0.26	0.29±0.19 *
sVCAM (μg/ml)	0.15±0.63	0.19±0.12
Cognitive testing		
Higher score is worse		
Number connection A (sec)	37.9±14.9	58.7±40.5 *
Number connection B (sec)	95.6±41.0	168±128 *
Line tracing time (sec)	100.7±35.6	117.0±57.0
Line tracing errors (number)	34.0±31.6	43.1±33.6
Serial dotting (sec)	64.6±21.8	89.9±33.7 *
ICT lures (number)	10.47±8.05	15.5±10.1 *
Lower score is worse		
Digit Symbol (raw score)	56.6±14.5	39.4±16.3 *
Block Design (raw score)	30.0±15.6	26.6±25.8
ICT targets (% correct)	96.24±6.26	87.4±13.7 *

* p<0.05 between groups, ICT: inhibitory control test, sVCAM: soluble vascular adhesion molecule, sICAM: soluble inter-cellular adhesion molecule

Table 3

Comparison according to etiology of cirrhosis

	Neither alcohol nor HCV (n=15)	Alcoholic liver disease alone (n=20)	HCV alone (n=30)	HCV+ alcoholic liver disease (n=25)
Age	57.7±5.7	52.7±8.4	56.7±5.2	55.9±4.3
Education (years)	14±2	13±6	13±6	13±5
Venous Ammonia (μmol/l)	38.6±27.6	48.2±22.6	55.7±32.6	61.9±26.1
MELD score	12.9±6.6	11.7±3.4	11.5±4.3	16.7±6.8 *
ADMA (μM)	0.49±0.21	0.41±0.12	0.40±0.12	0.57±0.19 *
IL-2 (pg/ml)	32.5±80.1	1.5±3.6	7.3±18.3	20.7±69.1
IL-1b (pg/ml)	27.4±75	1.7±4.2	5.9±15.1	4.3±10.5
TNF-alpha(pg/ml)	16.5±42.7	6.4±3.5	9.8±17.4	5.0±3.0
IL-6 (pg/ml)	43.2±69.9	5.4±6.2	12.9±33.5	22.9±44.7
IL-17 (pg/ml)	24.9±73.2	4.6±6.3	7.5±20.8	5.6±9.1
IL-23 (pg/ml)	748±2130	641±1370	1213±3432	1711±4254
IL-4 (pg/ml)	40.4±120.8	9.4±26.2	20.7±102.6	8.1±19.7
IL-10 (pg/ml)	10.9±26.9	3.8±8.9	8.8±27.1	4.5±6.2
s100b (pg/ml)	53.8±58.0	36.1±29.8	59.4±160.7	48.4±36.9
sICAM (μg/ml)	0.32±0.29	0.24±0.08	0.26±0.12	0.37±0.34
sVCAM (μg/ml)	0.16±0.74	0.15±0.09	0.18±0.07	0.21±0.17
Cognitive testing				
Higher score is worse				
Number connection A (sec)	47.2±28.5	46.4±29.2	40.1±23.7	51.6±25.6
Number connection B (sec)	131.8±91.8	122.7±156.4	115.3±81.6	152.8±100.0
Line tracing time (sec)	111.6±33.1	110.3±38.0	106.7±37.4	113.5±34.1
Line tracing errors (number)	39.4±29.6	31.4±28.8	31.4±28.8	50.8±35.3
Serial dotting (sec)	70.3±29.2	75.6±36.0	74.2±27.6	93.4±25.2
ICT lures (number)	14.0±10.8	10.7±7.4	9.9±8.9	21.7±7.5 *
Lower score is worse				
Digit Symbol (raw score)	49.5±15.8	53.4±18.2	52.8±16.6	32.2±11.0 *
Block Design (raw score)	22.7±12.4	22.0±15.5	30.4±15.6	16.9±9.3 *
ICT targets (% correct)	90/1±13.5	92.6±15.0	96.0±6.3	84.1±12.0 *

* P<0.05, Patients with both HCV and alcoholic cirrhosis had a significantly higher MELD and ADMA and worse cognitive performance on some tests compared to the other groups. ICT: inhibitory control test, sVCAM: soluble vascular adhesion molecule, sICAM: soluble inter-cellular adhesion molecule