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ORIGINAL ARTICLE

Predictive value of induced hyperammonaemia and neuropsychiatric profiling in relation to the occurrence of post-TIPS hepatic encephalopathy

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Abstract

Hepatic encephalopathy (HE) occurs in 20–50% of patients after transjugular intrahepatic portosystemic shunt (TIPS) placement. Older age, HE history and severe liver failure have all been associated with post-TIPS HE but it remains difficult to identify patients at risk. The aim of the present pathophysiological, pilot study was to assess the role of induced hyperammonaemia and associated neuropsychological and neurophysiological changes as predictors of post-TIPS HE. Eighteen TIPS candidates with no overt HE history (56 ± 8 yrs., MELD 11 ± 3) underwent neurophysiological [Electroencephalography (EEG)], neuropsychological [Psychometric Hepatic Encephalopathy Score (PHES) and Scan tests], ammonia and sleepiness assessment at baseline and after the induction of hyperammonaemia by an oral amino acid challenge (AAC). Pre-AAC, 17% of patients had abnormal EEG, 5% abnormal PHES, and 33% abnormal Scan performance. Post-AAC, 17% had abnormal EEG, 0% abnormal PHES, and 17% abnormal Scan performance. Pre-AAC, ammonia concentrations were 201 ± 73 μg/dL and subjective sleepiness 2.5 ± 1.2 (1–9 scale). Post-AAC, patients exhibited the expected increase in ammonia/sleepiness. Six months post-TIPS, 3 patients developed an episode of HE requiring hospitalization; these showed significantly lower pre-AAC fasting ammonia concentrations compared to patients who did not develop HE (117 ± 63 vs. 227 ± 57 μg/dL $p = 0.015$). They also showed worse PHES/Scan performance pre-AAC, and worse Scan performance post-AAC. Findings at 12 months follow-up ($n = 5$ HE episodes) were comparable. In conclusion, baseline ammonia levels and both pre- and post-AAC neuropsychiatric indices hold promise in defining HE risk in TIPS candidates with no HE history.

Keywords Cirrhosis · Ammonia · Oral amino acid challenge · Prognosis

Introduction

Hepatic encephalopathy (HE) is defined as a continuum of neuropsychiatric impairment caused by liver insufficiency and/or portal-systemic shunting (Sherlock et al. 1954; Parsons-Smith et al. 1957; Rikkers et al. 1978; Amodio et al. 2004; Montagnese et al. 2012; Vilstrup et al. 2014), ranging from abnormalities detected on neuropsychological/neurophysiological testing (minimal HE) to coma. The syndrome is thought to be largely due to neurotoxic substances of intestinal origin, for example ammonia, which are not cleared by the failing liver (Ong et al. 2003).

An additional cause of hyperammonaemia is upper gastrointestinal bleeding, in itself a complication of cirrhosis, because the blood-derived ingested amino acids are rapidly absorbed by the small intestine and oxidised to generate ammonia (Maier et al. 1979). This condition can be induced by the administration of a mixture of amino acids simulating the composition of blood (Balata et al. 2003; Al Mardini et al. 2006; Shawcross et al. 2007; Mardini et al. 2008, 2011; Casula et al. 2015) or glutamine (Oppong et al. 1997; Irimia et al. 2013). In the former case, the peak of ammonia is usually reached around 120–180 min after the ingestion, and accompanied by subjective sleepiness, electroencephalographic (EEG) slowing (Phillips et al. 1952; McDermott and Adams 1954; Sherlock et al. 1954; Douglass et al. 2001; Al Mardini et al. 2006; Bersagliere et al. 2012; Bersagliere et al. 2013) and neuropsychological abnormalities (Douglass et al. 2001; Mardini et al. 2008; Irimia et al. 2013).

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HE is also a common complication of transjugular intrahepatic portosystemic shunt (TIPS) placement, a procedure used to decrease portal pressure, and thus to manage gastrointestinal bleeding and refractory ascites. The likelihood of developing HE after TIPS ranges from 20 to 50%, with a peak at three months (Rossle et al. 1994; Sanyal et al. 1994; Somberg et al. 1995; Riggio et al. 1996; Nolte et al. 1998). The most important predictors for the development of post-TIPS HE are a history of overt HE (Riggio et al. 2010), older age (Riggio et al. 1996; Rössle et al. 1997), low portacaval gradient (< 10 mmHg) (Riggio et al. 1996), and severe hepatic failure (Riggio et al. 1996; Casado et al. 1998). Some studies suggest that hyponatraemia (Riggio et al. 2008; Guevara et al. 2010; Watson et al. 2019) and a history of minimal HE (Del Piccolo et al. 2002; Riggio et al. 2005) may also play a role.

The aim of the present study was to evaluate the predictive value of a large set of clinical, laboratory and neuropsychiatric parameters, both at baseline and after the induction of hyperammonaemia, in relation to the development of post-TIPS HE in a group of well-characterised TIPS candidates.

Patients and methods

Between January 2016 and May 2018, 32 patients were screened; four met one/more exclusion criteria (see below) and one did not wish to take part in the study. Therefore, a total of 27 consecutive patients (21 males; 56.4 ± 8.6 years of age) who had already been qualified as eligible for TIPS because of refractory ascites ($n = 18$, 67%), hydrothorax (3, 11%) or gastrointestinal bleeding (6, 22%) were prospectively enrolled. The aetiology of cirrhosis was alcohol in 14 patients (52%), viral in 9 (33%) and other in 3 (15%). Fifty-two % of patients were classed as Child A, 33% as B and 15% as C; average MELD score was 12 ± 3. Patients were excluded if they had misused alcohol in the preceding 6 months (documented by an accurate interview confirmed by a relative), had severe respiratory (link: https://www.sciencedirect.com/topics/medicine-and-dentistry/respiratory-failure), renal or heart failure (they had already been qualified as eligible for TIPS according to local protocols), less than 5 years formal education ($n = 1$ excluded), significant neurological or psychiatric comorbidity ($n = 2$ excluded) or were on psychoactive drugs ($n = 1$ excluded). The study protocol was approved by the Padua University Hospital Ethics Committee and conducted according to the 1975 Declaration of Helsinki (6th revision, 2008) and Good Clinical Practice (European) guidelines. All participants provided written, informed consent.

Study design

Baseline and experimental study sessions were conducted on two consecutive days. On the first day (baseline) patients arrived at 8:00 am having had breakfast; after a detailed clinical interview they underwent neurophysiological and neuropsychological assessment (see below). The following day patients arrived at 8:00 am having had no breakfast; baseline ammonia and subjective sleepiness levels were recorded prior to and at regular intervals after the ingestion of an oral amino acid challenge (AAC). On the second day, neurophysiological and neuropsychological assessment were performed from 11:00 am, i.e. at the time of the expected ammonia peak.

Neurophysiological assessment

Patients underwent recording of spontaneous, wake, eyes-closed EEG activity by digital 21-channel cup, with the ground placed on Fpz and the reference on Oz (Brainquick 3200; Micromed, Mogliano Veneto, Italy). Impedance was kept below 5 kΩ and the signal was filtered in the range of 0.33–60 Hz. Sampling frequency was 256 Hz and conversion resolution 0.19 mV/digit. After visual inspection to exclude artefacts, the EEG was assessed by spectral analysis on the bi-parietal derivation P3-P4; the mean dominant frequency (MDF) and the relative amount of slow EEG activity within the θ and δ frequency bands were calculated (Van der Rijt et al. 1984; Amodio et al. 1999). EEG abnormalities were classed as follows: relative theta power ≥ 35% with MDF > 7.3 Hz (grade I), MDF ≤ 7.3 Hz with relative delta power < 44% (grade II), MDF ≤ 7.3 Hz with relative delta power ≥ 44% (grade III) (Amodio et al. 1999); grade I, II and III were all considered abnormal (Van der Rijt et al. 1984; Amodio et al. 1999).

Neuropsychological assessment

Paper&pencil neuropsychological performance was assessed using the Number Connection Tests A and B, the Digit Symbol test, the Line Tracing test and the Serial Dotting test (Weissenborn et al. 2001). Results were scored in relation to age-adjusted and education-adjusted Italian norms (Amodio et al. 2008). Performance was classified as impaired if the sum of the standard deviations for the individual tests, referred to as the Psychometric Hepatic Encephalopathy Score (PHES), was ≤ − 4 (Amodio et al. 2008); the mean of the z-scores for each subtest (MPZS) (Amodio et al. 2008) was also used for purposes of correlation analysis. To avoid learning effects, different versions of the PHES subtests (of which four, approved and validated versions are available; Weissenborn et al. 2001), were used in the two subsequent assessments.

Then patients underwent the computerized Scan package (Sternberg 1969; Amodio et al. 1998; Montagnese et al. 2012), which consists of a three-level set of tests.

Simple reaction time (sRT): the subject is asked to respond to a series of 30 visual stimuli (an asterisk on the

computer screen) by pressing the spacebar on the keyboard. The number of correct responses, expressed as a percentage of the total number of stimuli (accuracy), and the accuracy-adjusted, average sRT (ms) are calculated.
Choice reaction time (cRT): the subject is presented with either the number 1 or the number 3 on the computer screen (30 trials) and asked to press 1 or 3 on the keyboard, accordingly. The number of correct responses, expressed as a percentage of the total number of stimuli (accuracy), and the accuracy-adjusted, average cRT (ms) are calculated.
Scan reaction time (Scan): the subject is presented with 36 consecutive pairs of numbers, which may or may not have common digits. The subject is asked to press 1 on the keyboard if there are digits in common between the two numbers (i.e. 4983, 691) and 3 if there are not (i.e. 481, 7562). The number of correct responses, expressed as a percentage of the total number of stimuli (accuracy), and the accuracy-adjusted, average Scan (ms) are calculated, together with a summary time-accuracy z score [this was qualified as abnormal if <2 with reference to local age- and education-adjusted normative values (Amodio et al. 1998)].

Oral amino acid challenge (AAC)

The AAC consisted of a banana flavoured, 54 g amino acid mixture (Glycine, 4 g, L-Alanine 7 g, L-Serine 3 g, L-Threonine 3 g, L-Proline 3 g, L-Valine 6 g, L-Leucine 7 g, L-Phenylalanine 3 g, L-Tyrosine 1.25 g, LAspartic acid 3 g, L-Asparagine 2 g, L-Glutamic acid 2 g, LArginine 1.25 g, L-Histidine 4 g, L-Lysine 4.5 g). This mimicks the composition of 400 ml blood haemoglobin (Douglass et al. 2001; Casula et al. 2015). The amino acid mixture was dispersed in 50–100 ml of water and ingested over a period of 5–10 min.

Capillary ammonia concentrations

Capillary ammonia concentrations were measured on the fingertip by use of the Blood Ammonia Checker kit (Menarini Diagnostics, Firenze, Italy). The skin was punctured with a lancet, 20 μl of blood obtained by capillary action and immediately transferred to an *ad hoc* reagent strip. Ammonia concentrations were then measured by a reflectance meter (Huizenga et al. 1995; Casula et al. 2015).

Subjective sleepiness

Patients were asked to rate their subjective sleepiness using the Karolinska Sleepiness Scale, which ranges from 1 to 9 (1 = "very alert"; 3 = "alert"; 5="neither sleepy nor alert"; 7="sleepy but no effort to remain awake" and 9="very sleepy, fighting sleep, difficulty staying awake") (Åkerstedt and Gillberg 1990).

Transjugular intrahepatic portosystemic shunt (TIPS)

TIPS insertion was performed in the interventional radiology suite after obtainment of written informed consent. The procedure was executed by transjugular route and the portal vein was punctured by a Rösch-Uchida Transjugular Liver Access Set (Cook Medical). PTFE-coated Viator stents (GORE) were used in all patients. The target value of portal pressure gradient was 12 mmHg or, alternatively, a reduction of at least 20% from baseline. However, all efforts were made to maintain the shunt diameter as small as possible in order to limit the risk of encephalopathy. Thus, the final diameter of the shunt and the reduction of the portal pressure were left to the decision of operator, either a hepatologist or a liver radiologist who worked in partnership.

Follow-up

Patients were followed up for 12 months from TIPS placement in relation to the development of HE-related hospitalizations, death and/or transplantation. Results are presented at both 6 and 12 months post-TIPS placement.

Statistical analyses

This was a pilot, pathophysiological study, thus no *a priori* power calculations were performed. Results are expressed as mean ± SD, unless otherwise specified. Normality was tested for by the Shapiro–Wilks test. Differences between patients who did/did not develop HE after TIPS over the follow-up period were tested by the Student t test for normally distributed variables (i.e. pre-AAC: MPZS, PHES z score, sRT, Scan, Scan accuracy, Scan z score, MDF and alpha EEG indices; post-AAC: sRT, Scan, Scan z score, MDF, alpha and delta EEG indices) and by the Mann-Whitney U test for non-normally distributed variables (i.e. pre-AAC: sRT, cRT and accuracy of cRT, beta, theta and delta EEG indices, post-AAC: MPZS, PHES z score, cRT and accuracy of sRT, cRT and Scan, beta and theta EEG indices). The time-course of subjective sleepiness/ammonia levels post-AAC was assessed by repeated measures ANOVA, *post hoc* Tukey test. Proportions of abnormal vs. normal neuropsychiatric tests at baseline and post-AAC in individuals who did/did not develop HE after TIPS were compared by the Pearson's χ^2. Kaplan-Meier curves were also plotted for time-dependent HE-development analysis.

Results

Out of the 27 patients recruited, 18 underwent both assessments (baseline and post-AAC) and TIPS placement [13 (72%) for refractory ascites, 1 (6%) for hydrothorax and 4 (22%) for secondary prophylaxis of variceal bleeding due to ≥2 episodes of bleedings despite medical and endoscopic therapy]. Four patients did not undergo the procedure because of ensuing complications/contra-indications to TIPS placement over the waiting period, one declined and 4 missed the post-AAC assessment. Of the 18 patients with complete datasets, 12 (67%) had alcohol-related cirrhosis and 6 (33%) viral cirrhosis; 15 (83%) were classed as Child A and 3 (17%) as B; average MELD score was 11 ± 3 (Table 1).

At baseline, 5 out of 27 patients (18%) had abnormal EEG (three classed as grade I and two as grade II), 4 (15%) abnormal PHES, and 9 (35%) abnormal Scan. Thus, 15–35% would have been qualified as having minimal HE (MHE), depending on the criterion used. Of the 18 patients with complete datasets, 3 (17%) had abnormal EEG (one classed as grade I and one as grade II), 1 (5%) abnormal PHES, and 6 (33%) abnormal Scan (Table 1). Thus, 5–33% would have been qualified as having MHE, depending on the criterion used. Two patients had type II diabetes (one was on dietetic treatment and one on metformin) and their neuropsychiatric performance was comparable to that of the rest of the sample ($n = 16$) both at baseline and post-AAC.

Prior to AAC, average, fasting capillary ammonia concentrations were 201 ± 73 μg/dL and subjective sleepiness 2.5 ± 1.2 on a 1–9 scale. After AAC administration, patients exhibited the expected increase in ammonia concentrations [$F(4, 56) = 2.7213$, $p = 0.038$; Fig. 1, panel A] and in subjective sleepiness [$F(4, 52) = 5.4002$, $p = 0.001$; Fig. 1, panel B]. After AAC, 3 out of 18 patients (17%) had abnormal EEG (all classed as grade I), none had abnormal PHES, and 3 (17%) had abnormal Scan. EEG results remained abnormal in two patients after AAC, normalized in one and became abnormal in one, while PHES normalised in the one patient who had abnormal PHES at baseline. Scan normalised in four patients, remained abnormal in one and changed from normal to abnormal in one. When neurophysiological and neuropsychological variables were treated as continuous, both the PHES (MPZS) and Scan (accuracy and overall z score) performance improved while the EEG, sRT and cRT remained substantially unchanged (Table 2).

All patients underwent TIPS without complications. In 16/18 patients an 8 mm Viator PTFE stent was placed, whereas a 10 mm stent was used in the remaining two. Final dilatation of the stent was performed with a 6 mm non-compliant balloon in 15 patients, 7 mm in 2 and 8 mm in remaining one. Portal pressure gradient was 19 ± 4.5 and 11.1 ± 1.9 mmHg pre and post stent insertion, respectively.

Follow-up analyses

Six months after TIPS placement, 3 patients had had an HE-related hospitalization (average time of onset 4 ± 2 months; Table 1). Compared to their counterparts who had not, these patients showed significantly lower, pre-AAC fasting capillary ammonia concentrations (117 ± 63 vs. 227 ± 57 μg/dL $p = 0.015$; Fig. 2, panel A). The time-course of capillary ammonia levels and subjective sleepiness prior to/after AAC in patients who did/did not develop HE are shown in panels A [$F(4, 52) = 2.5$, $p = 0.005$] and B [$F(4, 48) = 6.5$, $p = 0.0002$] of Fig. 3, respectively. Areas under the curve were significantly different in the two populations for both capillary ammonia levels and subjective sleepiness ($p = 0.003$ and $p = 0.04$, respectively). Patients who developed HE also showed worse Choice and Scan performance (in terms of accuracy) at baseline (Table 3). When baseline neuropsychiatric tests were qualified as normal/abnormal in relation to standard local thresholds, only a baseline abnormal PHES was associated with an increased risk of developing post-TIPS HE (33 vs. 0% in patients who did/did not develop HE, respectively; $\chi^2 =$

Table 1 Demographic and liver failure indices in all patients and by post-TIPS hepatic encephalopathy-related hospitalization at 6 and 12 months

		Post-TIPS HE-related hospitalization at 6 months		Post-TIPS HE-related hospitalization at 12 months	
	Final sample (n = 18)	Negative (n = 15)	Positive (n = 3)	Negative (n = 13)	Positive (n = 5)
Age (years; mean ± SD)	55.6 ± 8.4	55.5 ± 8.5	56.5 ± 9.3	54.5 ± 8.4	58.4 ± 8.6
Sex (% males)	87	67	80	77	80
Educational level (years; mean ± SD)	10 ± 3	11 ± 3	8 ± 0	11 ± 4	10 ± 3
Child A/B/C (%)	83/17/0	20/80/0	0/100/0	23/67/0	0/100/0
MELD score (mean ± SD)	11 ± 3	11 ± 3	11 ± 3	11 ± 3	11 ± 2
Etiology (% alcohol/viral/other)	67/33/0	73/27/0	33/67/0	61/39/0	60/40/0

HE, hepatic encephalopathy; *MELD*, Model for End Stage Liver Disease; *TIPS*, Transjugular intrahepatic portosystemic shunt

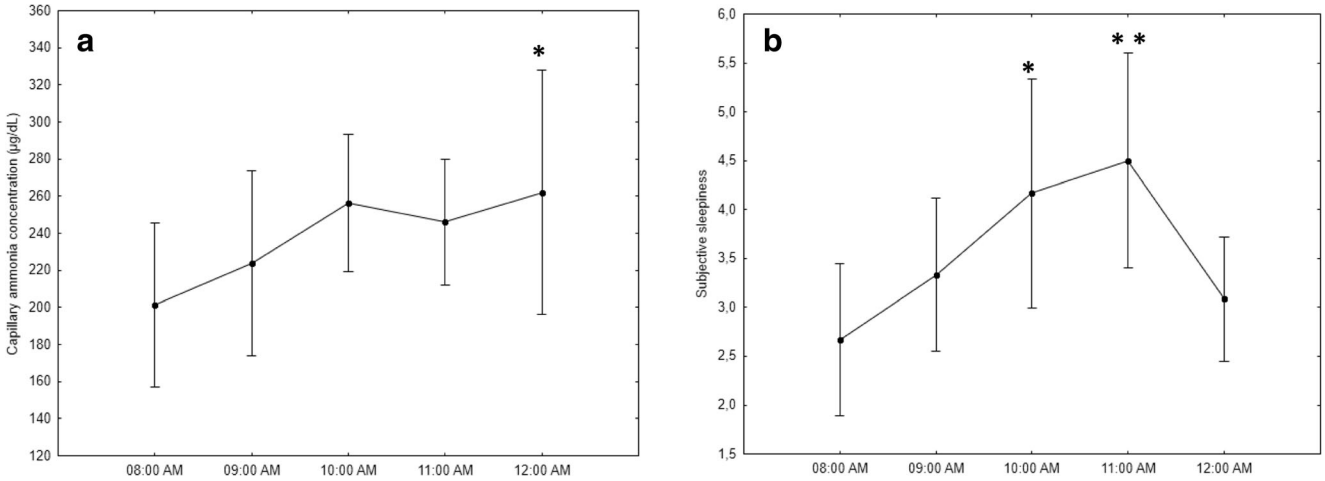

Fig. 1 Capillary ammonia concentrations (panel A) and subjective sleepiness (panel B) prior to (08:00 am) and after (09:00, 10:00, 11:00 and 12:00 am) the oral amino acid challenge (AAC). *Panel A: F(4, 56) = 2.7, p = 0.038. Panel B: F(4, 52) = 5.4, p = 0.001. * p < 0.05, ** p < 0.01* post hoc

5.3, $p = 0.02$). Patients who developed HE showed slower EEG (i.e. higher delta power) and worse Scan performance after AAC (Table 3). When post-AAC neuropsychiatric tests were qualified as normal/abnormal in relation to standard local thresholds, only a post-AAC abnormal Scan was associated with an increased risk of developing post-TIPS HE (67 vs. 7% in patients who did/did not develop HE, respectively; $\chi^2 = 6.5$, $p = 0.01$).

In relation to the occurrence of HE and characteristics of TIPS procedure, 2 of the 3 patients who developed HE within 6 months had a 8 mm stent dilated to 6 mm and one a 10 mm stent dilated to 8 mm; portal pressure gradient after TIPS was comparable in patients who did and did not develop HE (12.1 ± 0.9 versus 11.3 ± 0.4 mmHg, n.s.).

Twelve months after TIPS, 5 patients in total had had HE-related hospitalizations (average time of onset 8 ± 5 months; Table 4). Compared to their counterparts who had not, these patients showed significantly lower, pre-AAC fasting capillary ammonia concentrations (143 ± 67 vs. 238 ± 52 µg/dL p = 0.015; Fig. 2, panel B). All patients showed an increase in both capillary ammonia [$F(4,44) = 2.3$, $p = 0.05$] (Fig. 4, panel A) and subjective sleepiness [$F(4,40) = 5.7$, $p = 0.001$] over time (Fig. 4, panel B). Areas under the curve were significantly different in the two populations for both capillary ammonia levels and subjective sleepiness ($p = 0.006$ and $p = 0.04$, respectively). Patients who developed HE showed worse cRT performance (in terms of accuracy) at baseline (Table 4).

Of the patients who developed HE-related hospitalization after 6 months (and within 12 months), 1 had a 10 mm stent dilated to 7 mm and 1 an 8 mm stent dilated to 7 mm; portal pressure gradient after TIPS was comparable in patients who did and did not develop HE over the 12 months follow up period (11.8 ± 0.7 versus 10.7 ± 0.9 mmHg, n.s.).

Due to the limited number of events over the follow-up period, time-dependent survival analysis was not considered reliable and is not presented.

Based on the observations on ammonia levels and HE development, patients were split into those under ($n = 5$) and over ($n = 13$) the fasting ammonia lower quartile (145 µg/dL). Those under the lower quartile threshold were more strongly affected by the AAC, exhibiting significantly more prolonged post-AAC Scan reaction times (1830 ± 249 vs. 1391 ± 164, $p = 0.003$), more abnormal post-AAC Scan z scores (−2.2 ± 1.5 vs. -0.4 ± 0.8, $p = 0.02$) and a trend for a worse post-AAC MPZS (−0.02 ± 0.19 vs. 0.78 ± 0.80, $p = 0.08$) compared to their counterparts with baseline ammonia levels over the lower quartile threshold.

Table 2 Neuropsychological and neurophysiological indices (mean ± SD) at baseline and post-AAC

		Baseline	Post-AAC
PHES (z score)		0.00 ± 0.75	0.55 ± 0.81***
sRT (ms)		369 ± 64	357 ± 88
sRT (accuracy, n/30)		29 ± 1	30 ± 0
cRT(ms)		545 ± 103	498 ± 87**
cRT (accuracy, n/30)		29 ± 1	30 ± 0
Scan (ms)		1624 ± 276	1525 ± 251
Scan (accuracy, %)		78 ± 15	88 ± 9***
Scan (z score)		−1.64 ± 1.44	−0.90 ± 1.20**
Spectral EEG index	MDF (Hz)	10.0 ± 2.0	9.9 ± 1.4
	δ (%)	9 ± 9	6 ± 3
	Θ (%)	28 ± 17	30 ± 18
	α (%)	43 ± 18	45 ± 16
	β (%)	21 ± 14	18 ± 10

cRT, choice reaction time test; *EEG*, Electroencephalogram; *MDF*, Mean Dominant Frequency; *PHES*, Psychometric Hepatic Encephalopathy Score; *sRT*, simple reaction time test

** *p < 0.01* *** *p < 0.001* on the comparison between baseline and post-AAC

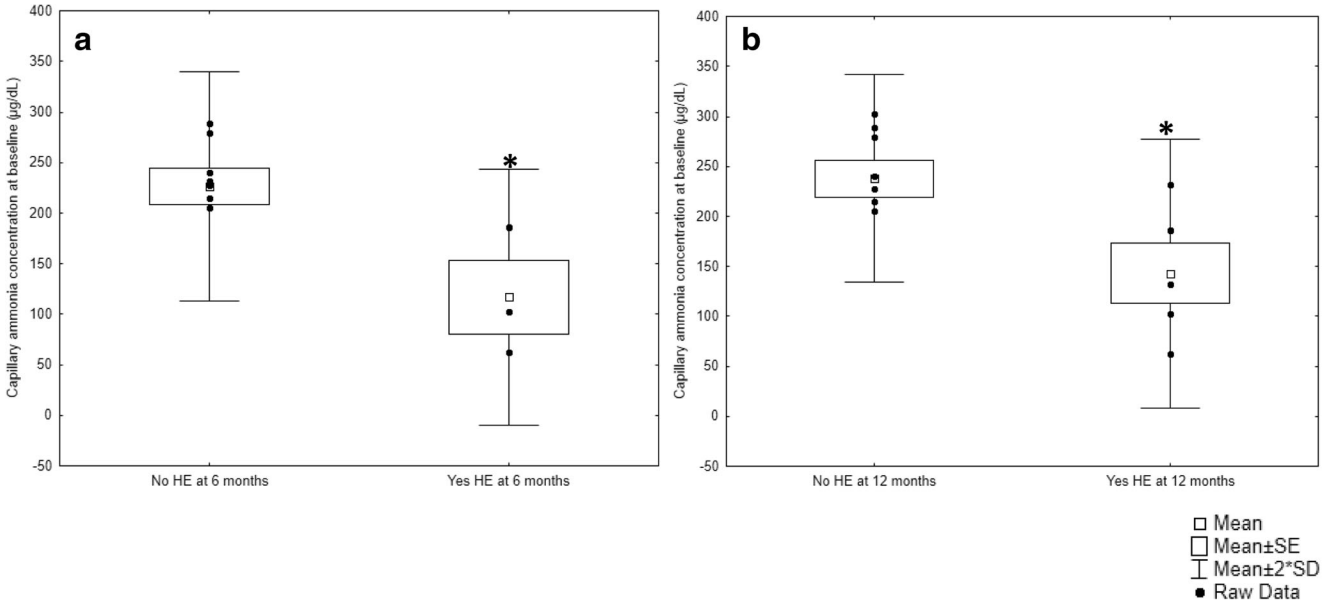

Fig. 2 Pre-AAC capillary ammonia concentrations in patients who did (right, $n = 3$) and did not (left, $n = 15$) develop an HE-related hospitalisation within 6 months of TIPS placement (panel A) and in patients who did (right, $n = 5$) and did not (left, $n = 13$) develop an HE-related hospitalization within 12 months of TIPS placement (panel B). * $p < 0.05$

Discussion

HE is a common complication of TIPS placement. Here we tested the predictive value of a large set of clinical, laboratory and neuropsychiatric parameters, at baseline and after the induction of hyperammonaemia, in 18 well-characterised TIPS candidates.

After AAC administration, both capillary ammonia concentrations and subjective sleepiness increased, in line with previous studies (Douglass et al. 2001; Bersagliere et al. 2012; Casula et al. 2015). As expected, capillary ammonia concentrations reached a peak at 120–180 min post-AAC (Douglass et al. 2001; Bersagliere et al. 2012). Neuropsychological performance tended to improve on the second assessment (180 min post-AAC), despite the AAC and the increase in ammonaemia, and in contrast with previous studies, which documented worsened neuropsychological performance post-AAC at 60 (Irimia et al. 2013) and 180 min (Oppong et al. 1997; Douglass et al. 2001). The post-AAC improved neuropsychological performance observed in this study, together with the absence of significant post-AAC EEG slowing (Douglass et al. 2001; Al Mardini et al. 2006;

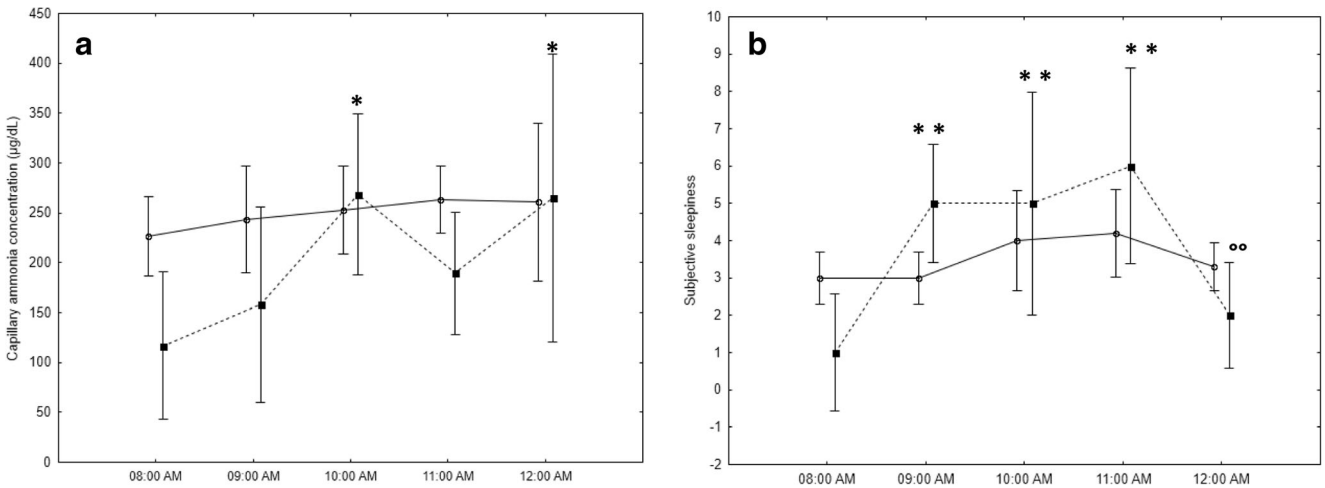

Fig. 3 Capillary ammonia concentrations (panel A) and subjective sleepiness (panel B) prior to (08:00 am) and after (09:00, 10:00, 11:00 and 12:00 am) the oral amino acid challenge (AAC) in patients who did (full squares and broken line; n = 3) and did not (empty circles and full line; n = 15) develop an HE-related hospitalization within 6 months of TIPS placement. *Panel A: group: F(1,13) = 2.1, p = 0.172; time: F(4, 52) = 5.16, p = 0.001; group*time: F(4, 52) = 2.5, p = 0.05. Panel B: group: F(1,12) = 0.1, p = 0.705; time: F(4,48) = 12.3, p < 0.0001; group*time: F(4, 48) = 6.5, p < 0.001. * p < 0.05, ** p < 0.01 on* post hoc *comparisons between pre-AAC (8:00) and post-AAC (09:00, 10:00, 11:00 and 12:00 am). °° p < 0.01 on* post hoc *comparisons between 11:00 and 12:00 am post-AAC*

Table 3 Neuropsychological and neurophysiological indices (mean ± SD) at baseline and post-AAC in patients who did/did not develop post-TIPS HE at 6 months

		Baseline		Post-AAC	
		No HE-related hospitalization (n = 15)	HE-related hospitalization (n = 3)	No HE-related hospitalization (n = 15)	HE-related hospitalization (n = 3)
PHES (z score)		−0.07 ± 2.25	−1.33 ± 2.52	1.27 ± 2.31	−0.67 ± 0.58
sRT (ms)		369 ± 70	369 ± 16	342 ± 84	431 ± 86
sRT (accuracy, n/30)		29 ± 0	28 ± 1	30 ± 0	30 ± 0
cRT(ms)		546 ± 101	542 ± 135	495 ± 87	513 ± 105
cRT (accuracy, n/30)		30 ± 0	28 ± 0*	30 ± 0	29 ± 1
Scan (ms)		1610 ± 275	1694 ± 333	1457 ± 185	1865 ± 299▪▪
Scan (accuracy, %)		82 ± 11	63 ± 24*	90 ± 6	76 ± 14▪
Scan (z score)		−1.44 ± 1.30	−2.60 ± 2.04	−0.56 ± 0.80	−2.59 ± 1.67▪▪
Spectral EEG index	MDF (Hz)	10.0 ± 1.9	10.1 ± 2.9	10.1 ± 1.4	9.0 ± 1.4
	δ (%)	8 ± 10	10 ± 6	6 ± 2	9 ± 3▪
	Θ (%)	27 ± 17	33 ± 22	28 ± 18	40 ± 19
	α (%)	44 ± 18	34 ± 15	47 ± 17	35 ± 15
	β (%)	20 ± 13	23 ± 18	19 ± 10	15 ± 6

cRT, choice reaction time test; *EEG*, Electroencephalogram; *MDF*, Mean Dominant Frequency; *PHES*, Psychometric Hepatic Encephalopathy Score; *sRT*, simple reaction time test

*$p < 0.05$ on the comparison no/yes-related hospitalization at baseline

▪$p < 0.05$ ▪▪ $p < 0.01$ on the comparison no/yes-related hospitalization post-AAC

Bersagliere et al. 2013), is most likely related to a mixture of patient selection (i.e. TIPS candidates with no history of HE and good liver function) and learning effect (i.e. patients obviously benefited from the repetition of the same tests over a short period of time). The former also justifies differences between the current and previous studies, which included a higher number of patients with MHE [5–33% vs. 80% (Irimia et al. 2013)] and with severe hepatic failure [i.e. Child C 0 vs.

Table 4 Neuropsychological and neurophysiological indices (mean ± SD) at baseline and post-AAC in patients who did/did not develop post-TIPS HE at 12 months

		Baseline		Post-AAC	
		No HE-related hospitalization (n = 13)	HE-related hospitalization (n = 5)	No HE-related hospitalization (n = 13)	HE-related hospitalization (n = 5)
PHES (z score)		−0.01 ± 0.72	0.02 ± 0.91	0.59 ± 0.83	0.45 ± 1.02
sRT (ms)		393 ± 73	369 ± 37	341 ± 89	399 ± 79
sRT (accuracy, n/30)		29 ± 1	29 ± 1	30 ± 0	30 ± 0
cRT(ms)		546 ± 109	543 ± 96	492 ± 92	512 ± 80
cRT (accuracy, n/30)		30 ± 0	29 ± 1 *	30 ± 0	30 ± 0
Scan (ms)		1613 ± 273	1653 ± 313	1467 ± 175	1675 ± 370
Scan (accuracy, %)		81 ± 11	73 ± 23	90 ± 6	85 ± 15
Scan (z score)		−1.5 ± 1.3	−2.0 ± 1.9	−0.6 ± 0.7	−1.5 ± 0.7
Spectral EEG index	MDF (Hz)	10.3 ± 2.1	10.0 ± 2.1	10.1 ± 1.5	9.3 ± 1.0
	δ (%)	9 ± 10	7 ± 6	6 ± 2	7 ± 4
	Θ (%)	28 ± 18	26 ± 18	29 ± 20	34 ± 16
	α (%)	41 ± 17	47 ± 22	45 ± 17	44 ± 16
	β (%)	21 ± 14	19 ± 14	20 ± 11	14 ± 5

cRT, choice reaction time test; *EEG*, Electroencephalogram; *MDF*, Mean Dominant Frequency; *PHES*, Psychometric Hepatic Encephalopathy Score; *sRT*, simple reaction time test

*$p < 0.05$ on the comparison no/yes-related hospitalization at baseline

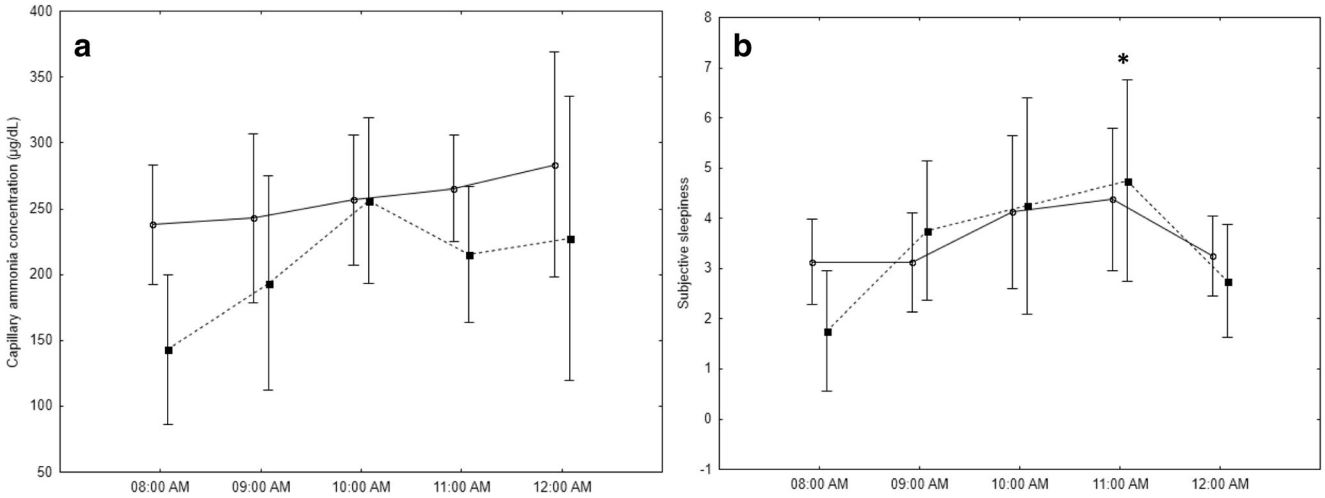

Fig. 4 Capillary ammonia concentrations (panel A) and subjective sleepiness (panel B) prior to (08:00 am) and after (09:00, 10:00, 11:00 and 12:00 am) the oral amino acid challenge (AAC) in patients who did (full squares and broken line; $n = 5$) and did not (empty circles and full line; $n = 13$) develop an HE-related hospitalization within 12 months of TIPS placement. *Panel A: group: F(4,44) = 2.3, p = 0.05*

7–32% (Douglass et al. 2001; Mardini et al. 2008; Irimia et al. 2013)]. By contrast, these compensated patients reported significant post-AAC subjective sleepiness, thus reacting to the AAC like healthy volunteers who have never experienced hyperammonaemia rather than like patients with cirrhosis with high baseline ammonia levels (Bersagliere et al. 2012), who have probably developed some degree of habituation. Also in line with this reasoning, patients who developed HE at six and 12 months post-TIPS placement showed lower pre-AAC capillary ammonia levels. Patients who developed HE at six months also showed higher post-AAC subjective sleepiness compared to their counterparts who did not develop HE. These findings suggest that compensated TIPS candidates (i.e. individuals who are by definition at low risk of HE and with good residual liver function) may encompass two different populations: 1) those who are well and have not had overt HE because their ammonia levels are near-normal, 2) those who are well and have not had overt HE despite hyperammonaemia, either because of brain habituation or some degree of subjective resistance to the development of the HE phenotype, for example in relation to higher cognitive reserve (Amodio et al. 2017). Thus the patients in this second group seem at lower risk of post-TIPS HE compared to those in the first group. Should habituation be the main driver of the observed differences, it would be possible to imagine habituation protocols based on the induction of mild hyperammonaemia by *ad hoc* challenges. While we have limited, anecdotal experience in this respect, we are indeed under the impression that subsequent challenges are better tolerated than the first one. This, however, may have alternative explanations, including familiarization with the procedures.

Besides ammonia levels and subjective sleepiness, also a number of neurophysiological and neuropsychological indices (some at baseline and some post-AAC) helped identifying patients who went on to develop post-TIPS HE. At baseline, PHES performance and cRT accuracy were worse in patients who went on to develop post-TIPS HE at both 6 and 12 months, respectively. This is in line with the knowledge that minimal/covert HE is a risk factor for overt HE both in general (Montagnese et al. 2011) and after TIPS (Berlioux et al. 2014; Nardelli et al. 2016). Post-AAC, the EEG was slower and Scan performance worse in patients who went on to develop post-TIPS HE at 6 months. These results confirm the potential usefulness of induced hyperammonaemia plus comprehensive neuropsychiatric evaluation in studying patients prior to TIPS placement. However, this type of assessment also has limitations: 1) it is time-consuming, 2) it requires experience and an adequate set-up, 3) it probably lends itself to oscillations in neuropsychiatric performance depending on the tests utilized and the time distance between baseline and post-AAC assessments, 4) its added value compared to a simple screening for the presence of minimal/covert HE has not been fully established within the context of TIPS placement. The present study is also not definitive in this respect. Therefore, the observation that lower pre-AAC capillary ammonia levels also identified patients at risk seems promising for purposes of general application. In addition, patients with lower ammonia levels seemed to tolerate the AAC less well, although the power of this study does not really allow to take all variables into account at once. Future, larger studies are needed to confirm these results, and especially to determine a useful capillary or venous ammonia threshold. Once this has been identified, it may be useful to assess the value of AAC within the group of patients with lower baseline ammonia levels. Finally, as the original study in which the AAC was first proposed (Douglass et al. 2001) documented significant neuropsychological and neurophysiological changes only in Child B and C patients (as opposed to Child A patients and

controls), the role of the degree of hepatic failure may need reassessment within the context of populations eligible for TIPS. Along similar lines, a pathological oral glutamine challenge was shown to be a prognostic factor for the development of overt HE in patients with MHE, while a normal oral glutamine challenge could exclude a significant risk of overt HE in those without MHE (Romero-Gómez et al. 2002).

In conclusion, baseline ammonia levels and both pre- and post-AAC neuropsychiatric indices hold promise in defining HE risk in TIPS candidates with no HE history.

Compliance with ethical standards

Conflict of interest None.

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