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QUALITATIVE AND QUANTITATIVE INDICES OF MILD HEPATIC ENCEPHALOPATHY

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Abstract

Background and Aims: While practices are becoming more homogeneous, the semi-quantitative assessment of overt hepatic encephalopathy (OHE) is not necessarily performed on a routine basis. The aim of the present study was to assess the reliability and impact of a qualitative approach to OHE diagnosis compared to the semi-quantitative, recommended one.

Methods: 411 patients evaluated in our dedicated HE clinic between April 2009 and June 2023 [292 males (71 %), 60 ± 10 years, MELD = 13.3 ± 5.0] were included. Patients were qualified as: 1) unimpaired, when they were clinically normal and both the Psychometric Hepatic Encephalopathy Score (PHES) and the electroencephalogram (EEG) were normal; 2) having covert HE when they were clinically normal but the PHES and/or the EEG were abnormal, or 3) having OHE based on the semi-quantitative modification of the West Haven criteria (Vilstrup et al., J Hep 2014). Patients were also classified as having/not having OHE based on a qualitative impression of the clinician, prior to any formal assessment. Based on the relationship between OHE assessments, they were further classified as True Positives (qualitative impression consistent with semi-quantitative OHE diagnosis), False Negatives (the physician missed OHE), False Positives (patients qualified as OHE did not meet the semi-quantitative criteria), True Negatives (neither the physician nor the formal criteria confirmed OHE).

Results: 137 (33%) patients were unimpaired, 201 (49%) had covert HE and 73 (18%) had overt HE; 122 (30% of the whole cohort) were qualified as having overt HE on qualitative assessment. Of the 73 patients with OHE on quantitative assessment, 19 (26%) were missed on qualitative assessment. In addition, 68 (20%) unimpaired/CHE patients (7 unimpaired/61 CHE) were wrongly qualified as having OHE by the physician. Similar neuropsychiatric profiles in terms of Animal Naming Test (ANT), PHES, quantitative indices of EEG slowing were observed in FN and TP. FNs had slightly worse neuropsychiatric performance compared to TNs.

Conclusion: Qualitative clinical evaluation of HE is not reliable, with significant proportions of both FNs and FPs. FPs were more commonly diagnosed with CHE, suggesting that physician was capable of detecting a mild degree of neuropsychiatric impairment. The diagnosis of OHE should be performed according to the semi-quantitative, recommended criteria.

Riassunto

Introduzione e scopo dello studio: La diagnosi semi-quantitativa dell'encefalopatia epatica conclamata (overt hepatic encephalopathy, OHE), seppur raccomandata dalle linee guida internazionali, non viene sempre eseguita nella pratica clinica. Lo scopo di questo studio è stato di valutare l'affidabilità e l'impatto di un approccio qualitativo rispetto a quello raccomandato.

Metodi: Sono stati inclusi 411 pazienti con cirrosi, valutati tra l'aprile 2009 e il giugno 2023 nell'ambulatorio specialistico dedicato all'encefalopatia epatica [292 maschi (71 %), 60 ± 10 anni, MELD = 13.3 ± 5.0]. Prima della valutazione formale, il medico ha indicato la presenza/assenza di OHE secondo la propria impressione qualitativa. I pazienti sono stati successivamente classificati come: 1) normali, se la valutazione clinica, la batteria Psychometric Hepatic Encephalopathy Score (PHES) e l'elettroencefalogramma (EEG) risultavano nella norma; 2) con encefalopatia epatica lieve (covert hepatic encephalopathy, CHE), quando erano clinicamente normali ma la PHES e/o l'EEG erano alterati, o 3) con OHE, secondo la versione semi-quantitativa dei criteri di West Haven (Vilstrup et al., J Hep 2014). In seguito, i pazienti sono stati ulteriormente classificati come: Veri Positivi, se l'impressione qualitativa coincideva con la diagnosi semi-quantitativa di OHE; Falsi Negativi, se il medico non aveva individuato la presenza di OHE; Falsi Positivi, se la presenza di OHE diagnosticata qualitativamente non coincideva con la diagnosi semi-quantitativa, e Veri Negativi, se

l'impressione clinica di assenza di OHE veniva confermata anche con i criteri diagnostici formali.

Risultati: 137 (33%) pazienti erano normali da un punto di vista neuropsichico, 201 (49%) presentavano CHE, e 73 (18%) presentavano OHE; 122 (30%) pazienti risultavano affetti da OHE sulla base della valutazione qualitativa del medico. 19 (26%) dei 73 pazienti con OHE non sono stati individuati alla valutazione qualitativa. Inoltre, 68 (20%) pazienti normali/con CHE (7/61) sono stati erroneamente qualificati come affetti da OHE secondo la valutazione qualitativa. I pazienti FN e VP presentavano profili neuropsichici simili, in termini di Animal Naming Test (ANT), PHES e indici di rallentamento dell'EEG (analisi spettrale). I profili neuropsichici dei FN erano lievemente peggiori rispetto a quelli dei VN.

Conclusioni: La valutazione qualitativa dell'encefalopatia epatica non è affidabile, in quanto si accompagna ad una quota significativa sia di FN che di FP. La maggior parte dei FP aveva una diagnosi formale di CHE, suggerendo che il medico era stato capace di individuare la presenza di un lieve disturbo cognitivo. La diagnosi di OHE deve formulata in base ai criteri semi-quantitativi raccomandati nelle linee guida nazionali ed internazionali.

1. Introduction

Hepatic Encephalopathy (HE) is a brain dysfunction, characterized by a wide spectrum of neuropsychiatric abnormalities, ranging from subclinical alterations to coma, caused by liver failure and/or portal-systemic shunting (1-3), and leading to higher blood levels of ammonia. HE is a significant complication of severe acute or chronic liver insufficiency, and/or of portal-systemic blood shunting (1-3).

The joint American-European guidelines (2-3) highlight the importance of the causal relation: encephalopathies of different etiologies, which may occur as a result of similar mechanisms, do not fall under this definition (for example, those induced by isolated defects of liver metabolism, valproate-induced hyperammonemia, or infections from urease-producer bacteria). At the same time, patients with liver insufficiency may suffer from other types of neurological alterations not related to hepatic failure/shunting. In addition, encephalopathies of mixed etiologies can also occur (2).

HE is frequently a recurrent condition, leading to multiple hospitalizations (2–5) and consistently affecting the quality of life (QOL) (2,6) by undermining the performance of complex tasks (i.e. driving), the capability of earning, and the fulfillment of the role of the patient in the family and society (2,7,8).

1.1. Epidemiology, clinical and socioeconomic impact

A precise estimate of prevalence of HE among patients with cirrhosis is a challenging task, due to the variability of the clinical presentation, the use of different diagnostic tools, and the absence of specific symptoms, especially in its milder forms (9).

According to the 2014 EASL/AASLD guidelines, 30% to 45% of cirrhotic patients may present an episode of clinically significant HE (overt HE, OHE), with the 5-year incidence of the first OHE episode being 5-25%, depending on the underlying causes (3): according to a long-term follow up study conducted in Sweden, patients with alcoholic cirrhosis had a 1-year cumulative incidence of HE almost ten times higher than those affected by viral hepatitis, and twice the 10-years cumulative incidence. Similar values were found regarding cirrhosis of other etiologies (cryptogenic, metabolic, Primary Biliary Cholangitis) (10). The overall incidence of HE is higher in patients with transjugular intrahepatic portosystemic shunt (TIPS), as reported in a recent review (9).

A first episode of OHE is associated with a survival rate of 35% to 45% at 1 year, and a 40% 1-year cumulative risk of recurrence (6). Grades III-IV OHE correlate with an increased in-hospital/30-day mortality, regardless of other organ failures (11); OHE is an independent predictor of mortality in liver transplant candidates at 90 days in the waitlist (12).

OHE is the second most frequent cause of hospitalization in cirrhotic patients, resulting in a significant economic burden both to healthcare and patients/caregivers in the US (4); in Italy, OHE is the first cause of hospitalization and is responsible for the highest rate of repeated hospital admissions in cirrhotic patients (13). HE has also been associated with longer inpatient stay and higher risk of hospital readmissions, compared to patients with cirrhosis without HE (5). The risk of HE-related hospitalization and mortality are higher in patients with higher MELD score (14).

Considering covert HE (CHE, *vide infra*), its prevalence in cirrhotic patients may be as high as 85% in some case series; however, presence of CHE varies widely according to the severity of the underlying liver disease, ranging from less than 25% in Child A patients to more than 50% Child C patients (15). The presence of CHE is associated with worse prognosis, quality of life and overall survival (16,17), and also with an increased risk of developing OHE over time (18,19), with a 40% 1-year cumulative risk of OHE occurrence (20). Moreover, patients with CHE may suffer from poorer sleep quality, impairment of sleep-wake cycle, and an increase in falls and car accidents, ultimately leading to the loss of self-sufficiency. Thus, this condition represents a major burden for affected families/caregivers and healthcare systems (7,8,21).

1.2. Pathophysiology

Although the pathophysiology of HE remains not completely understood, it is widely accepted that the neuropsychiatric symptoms and cognitive impairment are the result of blood-delivered factors, mostly gut-produced, which circulate in the bloodstream at increased levels due to the decreased clearance capacity of the cirrhotic liver. These factors act both altering the permeability and/or the integrity of the blood-brain barrier (BBB) and spreading through the brain stimulating pathophysiological pathways (22).

Pathogenic factors:

- Hyperammonaemia plays a key role in the pathogenesis of HE. Ammonia is the end product of protein digestion, amino acid deamination and bacterial urease activity; under normal circumstances its blood concentration is regulated by the liver through the urea cycle and it naturally crosses the BBB. Several organs and mechanisms contribute to the development of hyperammonaemia [*Figure 1*]: liver failure and portal-systemic shunting, alteration of gut microbiota (2), nitrogen metabolism and urea cycle in the gut and urinary system (23,24), sarcopenia, fasting state (25–27), inflammatory systemic conditions (28), hyponatremia (29), alkalaemia (30). Hyperammonemia exerts various deleterious effects through multiple pathways in the brain, including cellular swelling, mitochondrial dysfunction, and interference with

neurotransmission and oxidative metabolism, by promoting the production of inhibitory neurosteroids (2). Plasma ammonia, in addition to its direct effect on the brain, is likely to be a marker of the presence of other toxic nitrogenous substances produced by the gut microbiota (2).

- An increase in inflammatory cytokines is also part of the process (2). The inflammatory condition, sustained and eventually aggravated by the inflamed liver, bacterial translocation and precipitating events results in BBB dysfunction and neuroinflammation: peripheral cytokines and ammonia activate the brain microglia, which, in turn, amplifies the inflammatory reaction and exacerbates the effects of hyperammonaemia (6,28). The BBB is also damaged, especially in patients with acute liver failure (ALF) and, less so, those with acute-on-chronic liver failure (ACLF) (2).
- Bile acids may play a role in increasing neuroinflammation: an experimental study demonstrated that bile acids can induce neuroinflammation via bile-duct ligation, consequently producing a condition of HE in rats (31); in patients with end-stage liver disease, bile acid blood levels are elevated due to defective enterohepatic circulation (31,32).
- The excretion of manganese, a co-factor for ammonia-removing enzymatic reactions, is also compromised in end-stage liver disease: an accumulation of manganese within the basal ganglia has been observed, potentially being responsible for psychomotor impairment (33).

- Zinc deficiency has been observed in patients with HE, and it is demonstrated that oral zinc supplementation can improve neurocognitive function in these patients (34).
- Elevated levels of intracellular lactate in the astrocytes are a consequence of hyperammonaemia and possibly of reduced oxidative metabolism; it provokes the swelling of the astrocytes, impacting on neurotransmission and cell metabolism (35).

Involved cell types:

- Alzheimer Type 2 astrocytosis is the main neuropathological feature in HE, a morphological alteration of the astrocytes due to the aforementioned mechanisms (36). Astroglial cells show nuclear pallor, swelling and margination of the chromatin pattern. The phenomenon is more severe in cerebral cortex, basal ganglia, and cerebellum (37).
- As mentioned before, pro-inflammatory activation of microglial cell is also commonly implicated in HE (38).
- There is increasing evidence that neuronal cell death can occur, probably as a consequence of neurodegeneration. It is observed generally in end-stage cirrhosis and following multiple coma episodes (22,36).

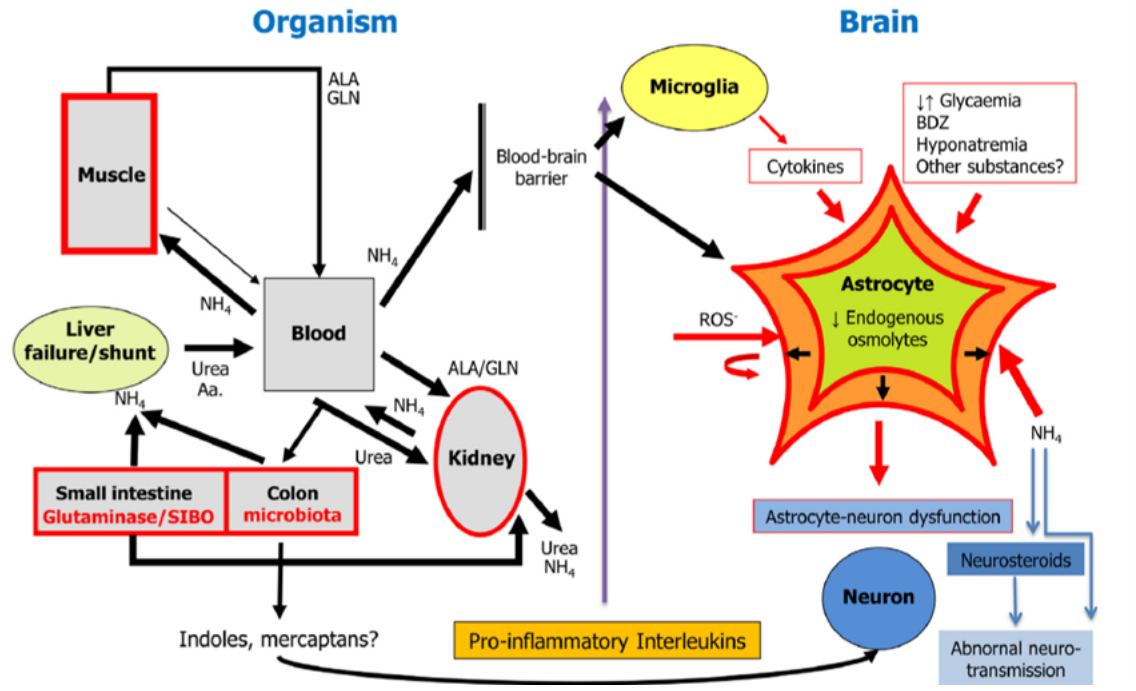

Figure 1: The pathophysiology of HE (2,39)

1.3. Classification

Classification is based on the underlying condition leading to HE: "type A" HE is due to ALF, "type B" HE is caused by portal-systemic shunt without significant liver disease, and "Type C" HE by cirrhosis, with or without portal-systemic shunting (2, 3). In terms of the extent of neuropsychiatric alterations, HE is qualified as *overt* (OHE) when patients are manifestly symptomatic, and graded (II-IV) according to the West Haven Criteria (2,3); *covert* (CHE), when patients are asymptomatic or present mild signs/symptoms, but they present alterations on neuropsychological and/or neurophysiological tests (2). Based on the time course, OHE can be qualified as:

episodic, as a single manifestation; recurrent, when two or more episodes occur in a 6-month span; persistent, in case of constant neuropsychiatric alterations (patients do not revert to normal or to baseline neuropsychiatric performance in between episodes) (2,3). Finally, HE is defined as *precipitated* if there is evidence of a known precipitating factor (3). Precipitating factors are infections, gastrointestinal bleedings, constipation, diuretic overdose, electrolytic disorders (2). Classification is summarized in Figure 2.

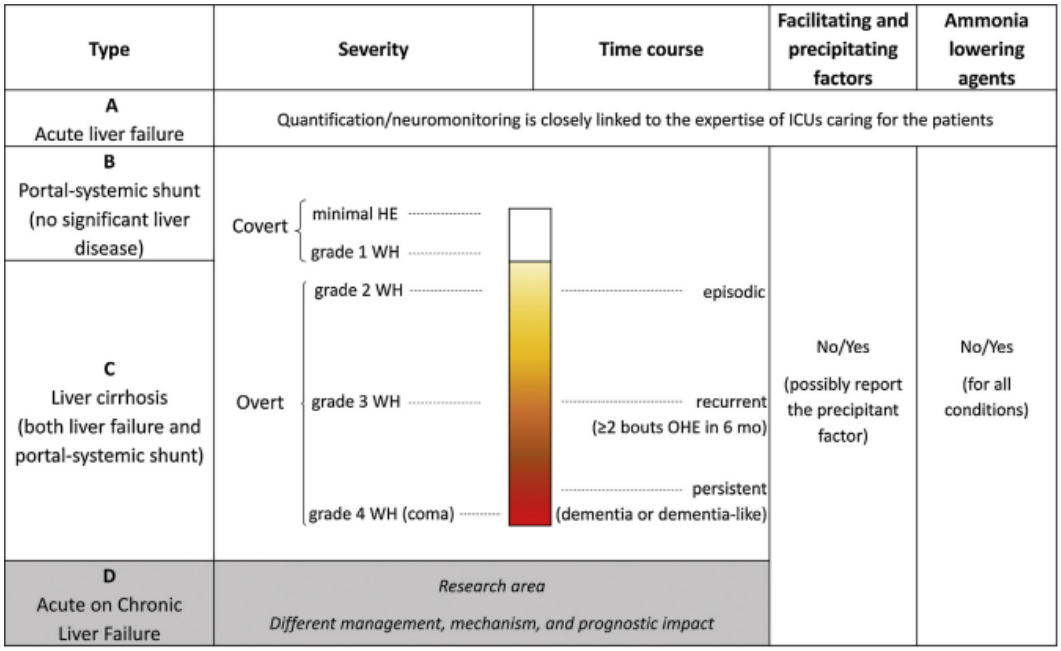

Figure 2: Classification of HE, from Montagnese et al. (2)

For this study, we took into consideration patients with type C HE, i.e., related with cirrhosis of the liver.

1.4. Diagnosis and differential diagnosis of type C HE

Although there are no specific manifestations nor clinical markers, the diagnosis of OHE is usually clinical: asterixis is the earlier and most frequent and symptom of OHE, alongside psychomotor slowing and disorientation on time (grade II HE according to the West Haven Criteria) and space (grade III HE). Other more severe and less commonly observed symptoms are the extrapyramidal syndrome, seizures, and lethargy until coma (3,6). It is essential to establish the presence of liver insufficiency and/or portal-systemic blood shunting, to rule out any other possible cause of the symptoms and to assess the severity of the condition (3): in this context, ammonia levels within the normal range have a high negative predictive value (40), thus, patients with overt neuropsychiatric abnormalities and normal ammonia levels should undergo a prompt differential diagnosis process, as they do not have a degree of liver failure and/or portal-systemic shunting that justifies a working diagnosis of HE (3, 40). Once a diagnosis of OHE is made, the identification and correction of possible precipitating events (that may coexist in the same patient) is crucial, as well as the managing of co-morbidities that can coexist (2, 3). Co-morbid conditions should always be considered, especially if neuropsychiatric symptoms do not ameliorate once the initial precipitant has been managed appropriately, thus brain imaging acquisition is recommended for differential diagnosis purposes (2); no cerebral imaging proves a diagnosis of HE (41).

In the circumstance of a difficult differential diagnosis, it has to be considered that plasma ammonia level does not have a diagnostic value and may remain elevated after clinical resolution of HE. On the contrary, it has a high negative predictive value, and correlates with the severity of HE (3,40,41). The electroencephalogram (EEG) typically presents triphasic waves and anterior-predominant abnormalities, which correlate with the severity of HE (42), in the context of a decelerated basic rhythmic activity. The cerebral MRI show spontaneous hypersignal in the basal ganglia in the T1 acquisition. However, it could reflect portal-systemic shunting more than HE (43). Performing MR spectroscopy can be useful to reinforce the diagnostic suspect, as it highlights a typical profile of the corona radiata (44). In addition, CT scan can be used in case MRI is not available or in the emergency setting.

For grading, West Haven Criteria and Glasgow Coma Scale are generally the methods of choice (41). In Italy, the latest guidelines recommend using an algorithm that combines West Haven Criteria, valuating the patient's orientation in space, time as well as the impairment of executive functions with the Animal Naming Test (ANT), and Glasgow Coma Scale (2). The algorithm is show in *Table 1*.

Table 1 - Algorithm for OHE grading, from Montagnese et al. (2)

1. Perform the Animal Naming Test (ANT)

Number of animals/min_____
If years of education < 8, please add 3 animals
If years of education < 8 and age > 80, please add 6 animals

> 15 animals (normal ANT) = No HE
< 15 animals (abnormal ANT), please go to 2

2. Orientation to time

	Incorrect	CORRECT
• What year is it?	☐	☐
• What season is it?	☐	☐
• What month is it?	☐	☐
• Which day of the month is it?	☐	☐
• Which day of the week is it?	☐	☐

At least 3 questions wrong = disoriented to time, please go to 3

3. Orientation to space

	Incorrect	CORRECT
• Which country are we in?	☐	☐
• Which region are we in?	☐	☐
• Which city are we in?	☐	☐
• Where are we? (church, park….)	☐	☐

At least 3 questions wrong = disoriented to space, please go to 4

4. Glasgow Coma Scale

Eye opening response

The patient does not open eyes (1 point)

The patient opens eyes in response to painful stimuli (2 points)

The patient opens eyes in response to voice (3 points)

The patient opens eyes spontaneously (4 points)

Verbal response

The patient makes no sounds (1 point)

The patient makes incomprehensible sounds (2 points)

The patient pronounces inappropriate words (3 points)

The conversation is confused, disoriented (4 points)

Motor response

The patient makes no movements (1 point)

Extension to painful stimuli (decerebrate response) (2 points)

Abnormal flexion to painful stimuli (decorticate response) (3 points)

Flexion/withdrawal to painful stimuli (4 points)

The patient localizes painful stimuli (5 points)

FINAL GRADING	
NO HE (grade 0)	ANT > 15 animals
Covert HE	Oriented to time Oriented to space ANT <15 animals
Overt HE grade II	Oriented to space Disoriented to time OR flapping tremor
Overt HE grade III	Disoriented to time Disoriented to space GCS = > 8
Overt HE grade IV (coma)	Disoriented to time Disoriented to space GCS < 8

In patients with cirrhosis and no history of OHE, screening for CHE should be performed with tests for which tools and local norms are available (41). The diagnosis of CHE cannot be based on the clinical presentation alone and requires testing, that can be neuropsychological (paper&pencil or computerized), neurophysiological and psychophysics (2, 41).

Neurophysiological

- The 24-derivation EEG, that can detect changes in cortical cerebral activity across the spectrum of HE; its reliability increases with quantitative (45,46). It requires institutional set-up, equipment, and expertise (2, 45);

Psychophysic

- The Critical Flicker Frequency (CFF) is the frequency, measured in Hz, at which a flickering light is indistinguishable from a steady light to the observer; studies show that it tends to decrease/improve following the state of the cognitive functions of the patient and the response to therapy. It also requires specialized equipment (47,48);

Neuropsychological

Neuropsychological tests, both using paper and pencil or computerized. The existence of pertinent local norms is crucial, as age and educational attainment are major confounders and need to be adjusted for. Computerised neuropsychological tests have the advantage of being based on repeated trials, thus the obtained average is

more stable than a single paper and pencil trial. On the other hand, they require familiarity with the device they are presented on (2).

Paper and pencil tests:

- PHES (Psychometric Hepatic Encephalopathy Score) is composed of five tests (the Number Connection Tests A and B, a Digit Symbol Test, the Serial Dotting Test and the Line Tracing Test [*Figure 3*]), and evaluates motor speed and accuracy, concentration, attention, visual perception and construction, visual-spatial orientation, memory; it has been proven to be of diagnostic as well as prognostic use, and predictive, in case of abnormal results, for both the occurrence of an episode of overt HE and survival (49–51);
- ANT (Animal Naming Test), a semantic fluency test that consists of listing as many names of animals as possible in one minute (52);

Computerized tests:

- CRT (Continuous Reaction Time) test, and particularly the CRTindex, assess the motor reaction time to auditory stimulus (the stability of them in case of the CRTindex) (53);
- Stroop test, which allows to test mental speed and flexibility. Patients have to identify the color of text words that spell a different color name (54);
- SCAN test is a computerized digit recognizing task which measures reaction time and percentage of errors (55);

- PVT (Psychomotor Vigilance Task) is a test of vigilance that measures the reaction speed to the appearance of consecutive numbers on the device's screen (56).

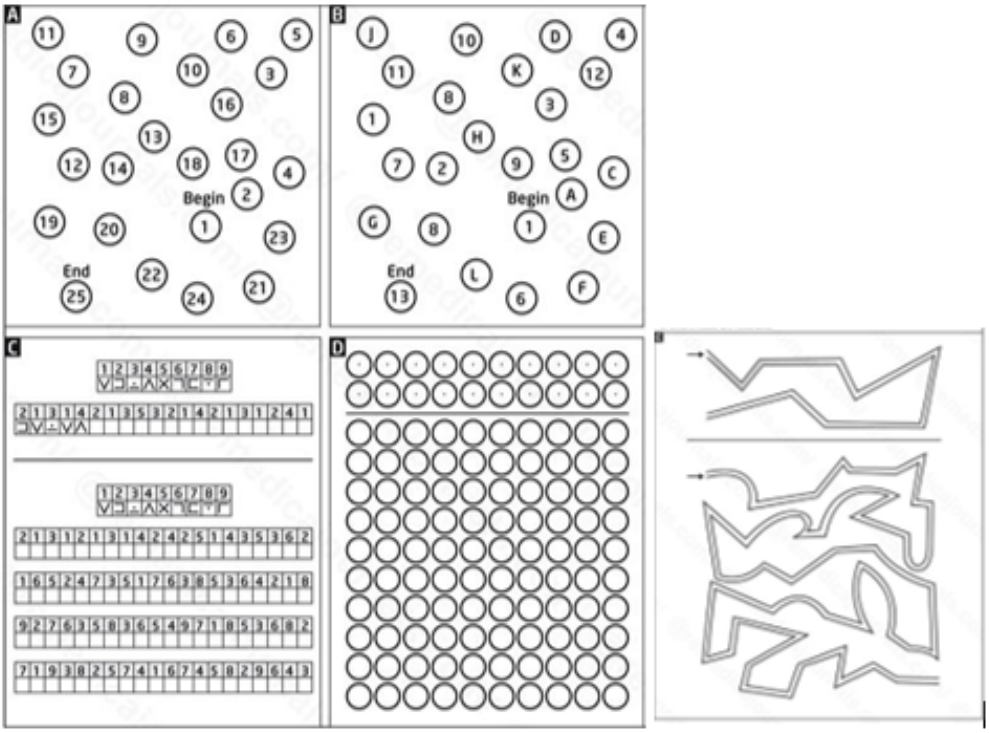

Figure 3 - Tests composing the PHES battery

1.5. Treatment

An episode of OHE, whether spontaneous or precipitated, should be actively treated. The managing of the episode primarily consists in pharmacological ammonia-lowering treatment and correction of precipitating factors (3). In the event of severe manifestation, it is also important to secure an adequate airway protection, with the possibility of using a nasogastric tube to administer oral therapies (3,41,56). Current therapies are valid options both for acute episodes and prophylaxis; after the first episode of HE, a secondary prophylaxis is indicated (41), as the likelihood of further ones is high and a common cause of hospital re-admission (13,58). The treatment options are:

- Nonabsorbable Disaccharides: lactulose and lactitol are used as the first-line treatment for HE. They act as osmotic laxatives and probiotics, with additional effects of reducing ammonia gut-absorption. Lack of effect of lactulose should prompt a clinical search for unrecognized precipitating factors and competing causes of brain impairment (3,41,59).
- Nonabsorbable Antibiotics: they act reducing endotoxemia and inflammation. Currently, rifaximin is the only antibiotic approved for HE treatment (59). Rifaximin is added to nonabsorbable disaccharides after the occurrence of a second OHE episode in a 6-month span, or in case non-absorbable disaccharides are not well tolerated (41). Rifaximin has been shown to significantly reduce the risk of

recurrence of HE and HE-related admissions (60). Despite having been considered an expensive treatment, reports have revealed that rifaximin administration is cost-effective, thanks to fewer HE-related admissions and shorter hospital course (61).

Furthermore, there is a wide selection of novel therapies that can be used. Non-ureic nitrogen scavengers, such as sodium benzoate, provide an alternative pathway for nitrogen disposal, and are mostly used in patients with urea cycle disorders. L-ornithine L-aspartate, nitazoxanide, acetyl-L-carnitine, probiotics, and branched-chain amino acids are also used with the purpose of decreasing the ammonia level (57). Albumin infusion may have a role in treatment and prevention of HE, due to its capacity of decreasing serum ammonia, inflammatory cytokines and endotoxins in the cirrhotic patient, as well as its anti-oxidative properties (57,62,63). Flumazenil may exert a negative modulatory effect on GABA-A receptor, reducing the GABA-A receptor complex's inhibitory effect on the postsynaptic neural membrane (64). It il also possible to regulate the composition and function of the gut microbiota through Fecal Microbiota Transplantation (FMT); recent studies show both a short and long-term impact on cognition, hospitalizations and HE recurrence (57). Highly recurrent or persistent OHE require a combination treatment associated with dietary changes (57).

In patients whose HE is related to TIPS, it is possible to reduce the width of the stent (65,66); In patients with large spontaneous portal-systemic shunts (SPSS), shunt embolization may be considered in selected cases (67).

The ultimate treatment for recurrent/chronic HE associated with a severely compromised quality of life is liver transplantation, as the condition is one of the exceptions to MELD for transplant access (57,68). In these cases differential diagnosis must be ruled out, as after-transplant neurodegenerative disorders will worsen (3).

2. Aim of the study

While a semi-quantitative assessment of overt hepatic encephalopathy (OHE) has been repeatedly and formally recommended (2,3), the impact of its replacement by unstructured interviews and/or physicians' impressions, which are still common practice, has never been formally assessed. The concept behind the recommendation is that verbal abilities tend to be preserved even in severe OHE (69,70), which can therefore be easily missed on conversation, unless questions ascertaining temporal and spatial orientation are actually asked.

The aim of the present study was to assess the reliability and the impact, if any, of a qualitative approach to OHE diagnosis compared to the semi-quantitative one recommended in the most recent set of Italian guidelines (2).

3. Patients and Methods

411 patients (71% males, 60±10 years of age) evaluated in our dedicated HE clinic between April 2009 and June 2023 were included. The clinic holds its evaluation in an outpatient setting, thus grade 4 patients were excluded a priori from the study.

Prior to any formal assessment, patients were classified as having/not having OHE based on a qualitative impression of the physician who clerked them into clinic, took their history and went on to examine them.

Patients were then formally evaluated by neuropsychological tests and electroencephalography (EEG), as fully described in Mangini et al., 2023 (71):

- neuropsychological evaluation was conducted using PHES test battery, composed by five paper and pencil tests (the Trail Making Test A and B, the Digit Symbol test, the Line Tracing test and the Serial Dotting test) (48,49). A PHES score ≤ −4, adjusted for age and education in relation to Italian norms, was considered abnormal (49);
- neurophysiological evaluation was conducted recording an EEG by 21-channels, 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 selection of an artifact-

free EEG section, spectral analysis was performed 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 (45,72). The EEG was considered abnormal if MDF was ≤7.3 Hz or if relative θ/ δ power was ≥35%/ ≥44% (45);

Referring to HE status on first evaluation, patients were qualified as:

- unimpaired, when they were clinically normal and both the psychometric hepatic encephalopathy score (also summarized as the so-called Mean PHES Z-Score (MPZS) (48,49)) and the EEG were normal;
- covert HE (CHE) when they were clinically normal but the PHES and/or the EEG were abnormal;
- OHE based on a recommended semi-quantitative modification of Conn's criteria (2). This includes orientation to time (5 questions) and space (4 questions) plus the Glasgow Coma Scale (GCS) (73). OHE was graded as II (oriented to space, disoriented to time or presence of asterixis), III (disoriented to time and space with a GCS ≥ 8) and IV (disoriented to time and space with a GCS < 8; coma) (2).

From 2016 onwards, the Animal Naming Test (ANT) was also administered (51).

The semi-quantitative and qualitative OHE diagnoses were compared, and patients qualified as true positives (TPs) when the impression of the physician was consistent

with the formal diagnosis of OHE; false negatives (FNs) when the physician did not recognize OHE; false positives (FPs) when patients were qualified as having OHE by the physician but they did not meet the formal diagnostic criteria; true negatives (TNs) when neither the physician nor the formal criteria confirmed an OHE diagnosis.

3.1. Statistical Analysis

Permission for retrospective data analyses was obtained from the local Ethics Committee.

Results are expressed as mean ± SD or as count and percentage, as appropriate. Comparisons were performed by ANOVA (*post hoc* Tuckey test) or by chi-square, as appropriate. Analyses were carried out with the package Statistica, version 13.1 (Dell, Round Rock, Texas, US).

4. Results

One hundred and twenty-two (30%) patients were qualified as having OHE on qualitative assessment.

On quantitative evaluation, 137 (33%) patients were qualified as unimpaired, 201 (49%) as having CHE, 57 (14%) as having grade II OHE, and 16 (4%) as having grade III OHE. Of the 73 patients with OHE, 19 (26%) were missed on qualitative assessment (FNs), with no difference in the likelihood of the physician missing grades II and III [*Table 2*]. Sixty-eight (20%) unimpaired/CHE patients were qualified as having OHE on qualitative assessment (FPs), of whom 61 (90%) had CHE and 7 (10%) were unimpaired [*Table 2*].

Demographic, hepatic failure and neuropsychiatric features of the patients, by agreement between quantitative and qualitative HE assessment are presented in *Table 3* and *Figure 4*. The latter (*Figure 4, panels B-D*) highlights how FNs had virtually identical neuropsychiatric profiles as TPs. By contrast, FPs had slightly worse neuropsychiatric performances than TNs, suggesting that the physician was probably capable of detecting CHE. Finally, while overall Model for End-stage Liver Disease (MELD) scores were low [*Table 3*], they were even lower in TNs [*Figure 4*].

Classes were homogeneous for sex, age, aetiology of the cirrhosis and MELD scores. There was a significant difference for what concerns asterixis (χ^2=145, p<0.001),

portal-systemic shunt (χ^2=13, p<0.05), and ammonia-lowering treatment (χ^2=18, p<0.001). As for ammonia levels, the difference was significant between TP and TN (p<0.001). The ANT test had a significant difference between TP and FP (p<0.05), FN and FP (p<0.05), TP and TN (p<0.001), FN and TN (p<0.01), FP and TN (p<0.05). The Mean PHES Z-score (MPZS) had as well a significant difference between TP and FP (p<0.05), FN and FP (p<0.001), TP and TN (p<0.001), FN and TN (p<0.01), FP and TN (p<0.01). The Mean Dominant Frequency (MDF) had a significant difference between TP and FP (p<0.01), FN and FP (p<0.05), TP and TN (p<0.001), FN and TN (p<0.01), FP and TN (p<0.001).

Table 2 - Relationship between qualitative and quantitative assessment of HE

Degree of hepatic encephalopathy	True Positive (TP)	False Negative (FN)	False Positive (FP)	True Negative (TN)	*Total*
Unimpaired, n (%)	0 (0%)	0 (0%)	7 (5%)	130 (95%)	*137*
Covert HE, n (%)	0 (0%)	0 (0%)	61 (30%)	140 (70%)	*201*
Overt HE, Grade II, n (%)	42 (74%)	15 (26%)	0 (0%)	0 (0%)	*57*
Overt HE, Grade III, n (%)	12 (75%)	4 (25%)	0 (0%)	0 (0%)	*16*
Total	*54*	*19*	*68*	*270*	*411*

Table 3 - Demographic, hepatic failure and neuropsychiatric indices, by agreement between quantitative and qualitative assessment of HE

		Available data (n)	All patients (n=411)	TP (n=54, 13%)	FN (n=19, 5%)	FP (n=68, 16%)	TN (n=270, 66%)
Sex (% males)		*411*	71	76	47	76	70
Age (years; mean±SD)		*411*	60±10	62±1	64±2	61±1	59±1
Aetiology (% alcohol/viral/metabolic/other)		*401*	34/30/6/30	24/40/6/30	47/16/5/32	43/25/7/25	34/31/5/30
MELD (mean±SD)		*318*	13.3±5.0	14.3±0.7	14.3±1	14.5±0.9	12.7±0.3
Ascites (% absent/mild/severe or refractory)		*370*	57/29/14	52/33/15	29/53/18	46/39/15	62/24/14
Asterixis (% absent/rare/frequent or continuous)$^{£££}$		*340*	79/12/9	19/41/40	41/35/24	80/13/7	93/6/2
Portal-systemic shunt (% absent/spontaneous/surgical)$^{£}$		*281*	45/49/6	30/56/14	23/77/0	48/50/2	49/45/6
Ammonia-lowering treatment (% yes/no)$^{£££}$		*385*	73/27	90/10	88/12	83/17	66/34
Ammonia (µmol/L; mean±SD)		*258*	67±44	96±10	67±7	73±6	59±3^{+}
ANT (mean±SD)		*300*	13±5	10±1	9±1	12±1$^{\wedge\,\$}$	15±1$^{+\,°°\,\#}$
MPZS (mean±SD)		*384*	-0.9±1.5	-2.1±0.2	-2.6±0.3	-1.1±0.1$^{\wedge\,\$\$\$}$	-0.5±0.1$^{+\,°°\,\#\#}$
Spectral EEG indices	MDF (Hz; mean±SD)	*383*	9.3±2.1	7.5±0.3	7.2±0.5	8.7±0.2$^{\wedge\wedge\,\$}$	10.0±0.1$^{+\,°°\,\#\#\#}$
	δ (%; mean±SD)		11±12	20±2	24±5	12±1$^{\wedge\wedge\,\$\$}$	9±0$^{+\,°°}$
	Θ (%; mean±SD)		35±19	49±2	47±4	42±2	29±1$^{+\,°°\,\#\#\#}$
	α (%; mean±SD)		36±18	21±2	20±3	31±2$^{\$\$}$	41±1$^{+\,°°\,\#\#}$
	β (%; mean±SD)		23±103	11±1	9±2	15±1	21±1$^{+\,°\,\#}$

$^{\wedge}$p<0.05, $^{\wedge\wedge}$p<0.01, $^{\wedge\wedge\wedge}$p<0.001, significance of the difference between TP and FP;
$^{\$}$p<0.05, $^{\$\$}$p<0.01, $^{\$\$\$}$p<0.001, significance of the difference between FN and FP;
$^{+}$p<0.001, significance of the difference between TP and TN;
$^{°}$p<0.05, $^{°°}$p<0.01, significance of the difference between FN and TN;
$^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001, significance of the difference between FP and TN
$^{£££}$Asterixis: ^{2}=145, p<0.001; $^{£}$Portal-systemic shunt: ^{2}=13, p<0.05; $^{£££}$Ammonia-lowering treatment: ^{2}=18, p<0.001

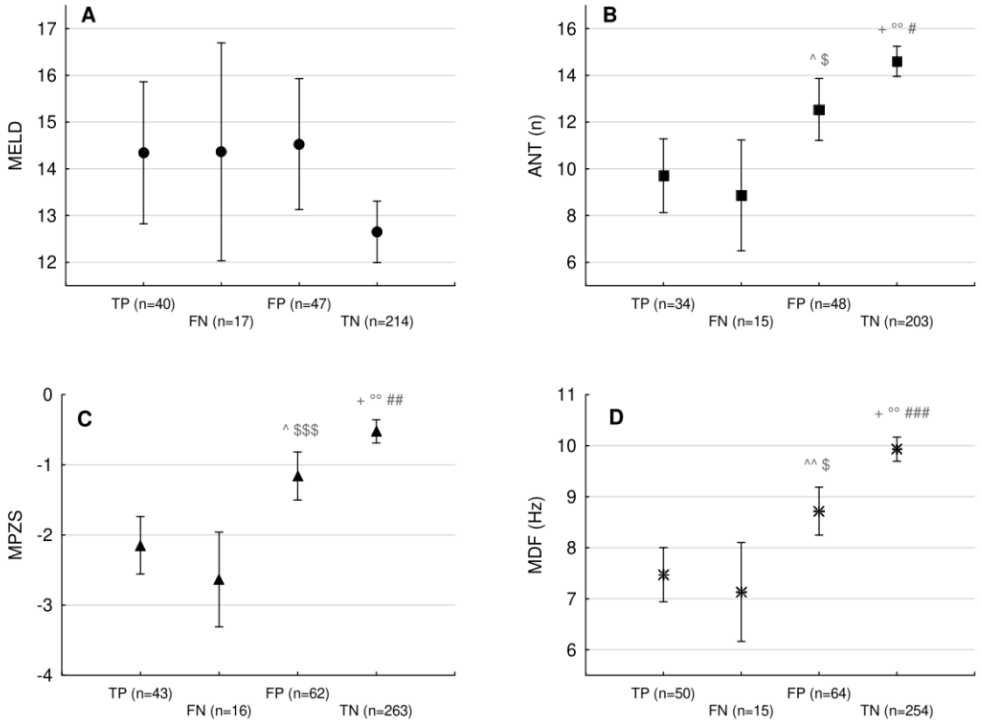

Figure 4 *- Mean ± 95% confidence interval of the variables Model for End-stage Liver Disease (MELD;* ***A****), Animal Naming Test (ANT, the higher the number of animals listed in 60 seconds, the better the performance;* ***B****), Mean PHES z Score (MPZS, the higher the z score, the better the performance;* ***C****), and the spectral EEG parameter Mean Dominant Frequency (MDF, the higher the frequency, the better the EEG;* ***D****) by classes of agreement between quantitative and qualitative assessment of hepatic encephalopathy; TP: true positives, FN: false negatives, FP: false positives and TN: true negatives.* $^{\wedge}p<0.05$, $^{\wedge\wedge}p<0.01$*, significance of the difference between TP and FP;* $^{\$}p<0.05$, $^{\$\$\$}p<0.001$*, significance of the difference between FN and FP;* $^{+}p<0.001$*, significance of the difference between TP and TN;* $^{\circ\circ}p<0.001$*, significance of the difference between FN and TN;* $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$*, significance of the difference between FP and TN on post hoc comparisons (Tukey test).*

5. Discussion

In this study, qualitative and quantitative evaluation of OHE were compared. In most outpatient contexts, HE is assessed with a single qualitative evaluation, based on the experience of the physician - even though a more precise examination is possible by performing a simple semi-structured interview, including a small set of questions, as recommended by the Italian guidelines [*Table 1*]. Qualitative clinical evaluation has been proven unreliable by the results of this study, with approximately a quarter of patients with grades II and III OHE being missed.

This confirms the appropriateness of the recommended evaluation tools, and most likely also the theory behind them. This evaluation is not time-consuming and requires less expertise compared to the extensive evaluation which is run in a tertiary referral center, as described above, and is indeed a very reasonable investment by comparison to the missed diagnoses of grades II/III OHE patients.

On the other hand, it is necessary to point out that the large majority (90%) of FP, had CHE. This finding suggests that a skilled physician is often capable of detecting a mild degree of neuropsychiatric impairment, which does not result in temporal disorientation or asterixis, thus not meeting the formal criteria for OHE diagnosis (2).

There were no differences regarding demographic indices: groups were homogeneous for age, sex or aetiology of the cirrhosis, meaning that none of these factors has been a bias nor has affected the qualitative evaluation of the physician. MELD was comparable between the groups, which was predictable, because the study has been conducted in an outpatient clinic which is part of a tertiary referral center for liver disease: for this reason, patients included were all suffering from chronic liver disease, but none of them at a stage that would prevent them from undergoing an outpatient evaluation, or that would require hospitalization. This also explains the great number of CHE in relation to those with OHE included in the study. The same applies to the presence of SPSS: this condition is one of the main mechanisms for the pathogenesis of recurrent/persistent HE (74), and is related to a higher risk of developing HE (75). SPSS are present in approximately 45-70% of patients with cirrhosis and recurrent or persistent HE (76). On the contrary, ascites and HE are two complications of cirrhosis that are not correlated; distribution of ascites is homogeneous because of this independency.

Differences in plasma ammonia levels between TP and TN groups confirms its negative predictive value (41). Even though ammonia plays a central role in the pathophysiology of HE (22), in the other groups ammonia levels do not correlate with HE grade, probably because of several reasons: a high inter-individual variability in the response to hyperammonemia (77,78); patients without HE can display hyperammonaemia (41); sample handling and processing impact ammonia levels

across sites (78); ultimately, ammonia analyses are often not systematically performed or timed (41).

The results of the tests used in this study for HE assessment (ANT, MPZS and spectral EEG indices) all shown significant differences between groups, highlighting the importance of a comprehensive evaluation for the diagnosis and grading of HE. It also seems that flaws of qualitative clinical evaluation of HE concern different aspects of cognitive impairment. In *Figure 4* it is evident that worse results, regarding ANT, PHES and EEG tests, in FP compared to TN also corroborate the assumption that an expert physician is often capable of detecting the presence of mild cognitive impairment.

Lastly, and as indicated by the significant difference of ammonia-lowering treatment percentages, patients with prominent symptoms are more likely to be already on ammonia-lowering therapy.

While the data on FPs may be confounded by the fact that the study was conducted in a tertiary referral Hepatology center with a research interest in HE, the data on FNs are even more worrying for the same reason: using the sole qualitative evaluation, even an expert physician, within the context of HE assessment, failed to identify roughly one patient suffering from OHE out of four. This, together with the fact that marks a worsening in both hepatic function and prognosis (2), accounts for the harmfulness of using the sole qualitative evaluation. The non-recognition of the condition translates primarily to the delay of treatment, possibly resulting in an

increasing of the number of hospitalizations (14). Moreover, the diagnosis of OHE is also fundamental for any subsequent neuropsychiatric evaluation for purposes of TIPS or liver transplant selection (67).

In clinical practice, the use of semi-quantitative and quantitative tools for the evaluation of psychological and neurological impairment is well established: neurologists, psychiatrists and geriatricians all need and use this kind of tools to assess their patients' performance (79–81). HE is by definition an ensemble of neuropsychiatric symptoms; as it is possible to understand from this study, a semi-quantitative evaluation is essential for the correct detection of the condition, the presence of which impacts heavily on patient's life quality and expectancy.

6. Limitations

This monocentric, retrospective study has some limitations, which are mainly intrinsic to the characteristics of the study itself: data on clinical history, especially related to previous HE episodes, which could have added information on the population, were not analyzed. The majority of patients were already on ammonia-lowering treatment, suggesting a selected population bias; moreover, this study has been conducted in a tertiary hepatology center with an expertise on HE, where clinicians have likely better experience to recognize HE patients. To confirm the results, a multicenter study would be recommended.

7. Conclusion

Qualitative OHE diagnosis is not reliable, with significant proportions of both FNs and FPs. Despite the fact that FPs were very commonly diagnosed with CHE, suggesting that physician was capable of detecting a mild degree of neuropsychiatric impairment, our data support the contention that the diagnosis of OHE should be performed in a semi-quantitative fashion, according to guidelines.

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