PH D THESIS - SUMMARY

STANDARDIZATION OF THE PSYCHOMETRIC HEPATIC ENCEPHALOPATHY SCORE IN A TERTIARY CARE CENTER FROM NORTHEASTERN ROMANIA

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LISTA ABREVIERILOR

AASLD - Asociația Americană pentru Studiul Bolilor Ficatului
BH - biopsie hepatică
CBP - ciroză biliară primitivă
CFF - frecvența critică intermitentă
CH - ciroză hepatică
CLDQ - chronic liver disease questionnaire
CRT - continuous reaction time test
CT - computer-tomografie
DST - digit-symbol test
EASL - Asociația Europeană pentru Studiul Ficatului
EEG - electroencefalografie
EH - encefalopatie hepatică
EHM - encefalopatie hepatică minimă
EHD - encefalopatie hepatică decompensată
GGT - gama glutamil-transpeptidaza
gmp - guanozin monofosfat ciclic
HCC - carcinom hepatocelular
HRQoL - calitatea vieții legată de sănătate
HVPG - gradientul presional venos hepatic
IL - interleukină
ICT - testul de control al inhibiției
IHA - insuficiență hepatică acută
LTT - line tracing test
LTT-e - line tracing test errors
LTT-t - line traging test time
MELD - Model for End Stage Liver Disease
MHE - membrana hemato-encefalică
NCT-A - number connection test A
NCT-B - number connection test B
NO - oxid nitric
PBS - peritonită bacteriană spontană
PE - potențiale evocate
PIINP - peptidul N-terminal al colagenu lui de tip III
RMN - rezonanță magnetică nucleară
SDT - serial dotting test
SRA - specii reactive de oxigen
SRO - specii reactive de oxigen
SIP - Sickness impact profile
SIRS - sindromul de răspuns inflamator sistemic
SNC - sistem nervos central
TGO - transaminaza glutamică-oxalacetică
TGP - transaminaza glutamică-piruvică
TIMP-1 - inhibitorul metalopeptidazei 1
VE - varice esofagiene
Introduction

Minimal hepatic encephalopathy (MHE) is a neuropsychiatric disorder with cognitive alterations diagnosed with neuropsychological and neurophysiological tests, in patients with chronic liver disease. MHE is a subclinical stage of HE and encompasses a broad spectrum of neurologic alterations: reduced attention, decreased inhibitory control and impaired executive functions (239,296).

MHE prevalence varies worldwide between 30 and 84% (146). The absence of standardized diagnostic criteria and the subtle clinical manifestations led to the underevaluation of MHE. This condition has a major negative impact on the quality of life and work capacity of a person, generating high expenses for both the individual and the society (170). MHE is associated with sleep disorders, memory and learning deficits, low socio-economic status, reduced driving capacity and a more rapid evolution towards overt hepatic encephalopathy (OHE). Also, MHE impairs daily activities like reading and social interactions (144).

Diagnosis and treatment of MHE are not recommended to all cirrhotic patients. Evaluation and initiation of MHE therapy are advised for patients with high risk of accidents (at work/while driving) and for individuals with symptoms of MHE or reduced work capacity (161).

Multiple neuropsychological and neurophysiological tests are used to diagnose MHE: PHES (Psychometric hepatic encephalopathy score), ICT (Inhibitory control test), CFF (Critical flicker frequency), CDR (Cognitive drug research), Stroop-Test, CRT (Continuous reaction time test), RBANS (Repeatable Battery for the Assessment of Neuropsychological Status). However, most of them are expensive and require advanced equipment or trained investigators in order to be applied (164).

The EASL/AASLD and the ISHEN guidelines recommend the PHES as a first line test for MHE diagnosis (33,163). The PHES is a paper-and-pencil test that encompasses 5 neuropsychometric evaluations: DST, NCT-A, NCT-B, SDT and LTT. The entire testing procedure can take place at the bedside or at a desk, without sophisticated equipment or trained personnel (165).

The PHES has been standardized in several countries: Germany, Italy, Spain, Portugal, Korea, China, Mexico, India, England, Poland, Taiwan and recently Cuba. According to previous studies, the test results can be influenced by multiple factors (age, occupation, education or sex) and the nomograms are different according to the tested population. The Romanian PHES validation would join the international effort to standardize a universal test with cross-cultural applicability (32,172,174-184).

Romania is an epidemic area for virus B and C hepatic infections. Moreover, in our country there is a high incidence of chronic liver diseases (297,298). In Romania, MHE is an underdiagnosed condition. There are no standardized psychometric tests that can be used to detect MHE and there is no information about the prevalence and epidemiologic characteristics of this disease in Romanian cirrhotic patients.
Chapter IX. Motivation and aims

Primary aims:

• standardization of the PHES for MHE diagnosis in a group of healthy volunteers from northeastern Romania
• estimation of MHE prevalence on a group of patients with liver cirrhosis from a tertiary care center in northeastern Romania

Secondary aims

• evaluation of PHES characteristics in a group of patients with liver cirrhosis
• comparison of the Romanian PHES results with the German PHES results

Study originality

This research aimed to validate the first psychometric tests for the diagnostic and evaluation of MHE characteristics in Romania. Also, it is the first study that estimates the prevalence of MHE in Romania. This investigation was justified by the growing interest for MHE in the recent years (126,130,139,164). This condition is considered a public health problem that severely impairs the quality of life in patients with liver cirrhosis (157). The diagnosis and evaluation of MHE in Romania could generate useful information the treatment and social understanding of this disease (family issues, work capacity, social support). The PHES is acknowledged worldwide as a first line test for the diagnosis of MHE. Literature studies use the PHES as a reference for the evaluation of other MHE diagnostic tests (164). PHES studies three main aspects of cognition (attention, concentration capacity, psychomotor abilities) and has a high sensibility and specificity for MHE diagnosis (165).

Ethics

All subjects that attended the study signed an informed consent that summarized the methodology, aims, benefits and risks of the study and also information about the confidentiality of the personal data.

The study protocol followed the ethical recommendations from the Declaration of Helsinki 1975 and was approved by the Ethics Committee of the hospital and the Ethics Counsel of the University of Medicine and Pharmacy "Grigore T. Popa" - Iasi.

This study was supported by the project “Excellence program in doctoral and postdoctoral research on chronic diseases”, contract number POSDRU/159/1.5/S/133377, recipient U.M.F. “Grigore T. Popa” - Iasi, cofinanced by the European Social Fund through the Sectorial Operational Program for Human Resources Development 2007-2013.
Chapter X. Material and methods

During my personal research I performed a prospective study that lasted 24 months (1st of January 2014 - 31 December 2015) in the Institute of Gastroenterology and Hepatology - Clinic Emergency Hospital "Sf. Spiridon" Iasi. The study involved 260 healthy subjects and 106 patients with liver cirrhosis.

Exclusion criteria:

A) Healthy volunteer group

- active alcohol drinking (> 50 grams/day - men, > 30 grams/day - women - for the last three months);
- chronic/acute renal or liver disease;
- history/recent diagnosis of neurologic/psychiatric conditions;
- neuropsychiatric active treatment;
- severe renal/heart/pulmonary conditions;
- visual disturbances not corrected by glasses;
- illiteracy;

B) Liver cirrhosis group

- presence of OHE (diagnosed according to West-Heaven criteria);
- history or diagnosis of hepatocellular carcinoma or other malignancies;
- recent spontaneous bacterial peritonitis or upper digestive bleeding;
- history of TIPS or porto-systemic shunt surgery;
- use of lactulose and/or antibiotics; active alcohol consumption (> 50 g/day - men or >30g/day - women - for the last three months);
- severe renal, cardiac, pulmonary or vascular disease;
- active use of psychotropic drugs or presence of neuropsychiatric disorders;
- visual disturbances not corrected by glasses;
- illiteracy;

Study protocol

A) Healthy volunteer group

The healthy subjects were relatives or close friends of the inpatients from the Institute of Gastroenterology and Hepatology Iasi. Each subject underwent a short interview regarding alcohol consumption, medical history and visual problems. To gather a representative group from the Romanian general population, patients were stratified according to age (18-40 years, 41-60 years, 61-80 years), sex and education years (8 years, 9-12 years and > 12 years).

B) Liver cirrhosis group

Patients without a previous diagnosis of HE inpatients of the IGH Iasi were included in the study. The individuals were diagnosed with cirrhosis using a combination of clinical examination, blood tests, imaging techniques, upper digestive endoscopy and noninvasive evaluation of liver fibrosis and/or liver biopsy.
In the day of the tests, blood was drawn from the liver cirrhosis patients for common laboratory analysis and venous ammonia. Liver function was assessed using the Child-Pugh and MELD scores.

All of the subjects (both healthy and cirrhotics) were interviewed about occupational status, ethanol consumption, knowledge of the Romanian alphabet and numbers. The data were recorded in an individual working sheet.

C) Psychometric tests

PHES encompasses 5 tests: NCT-A, NCT-B, DST, SDT and LTT. The median completion time of the five tests is 20 minutes. The use of a pencil is recommended (165).

After a previous demonstration, all of the subjects from the study completed the five tests according to the instructions provided by the investigator. The patients performed the tests between 10 a.m. and 3 p.m. at a desk (in a silent, well illuminated room). A trained investigator supervised and interpreted the tests.

The German normative data, the PHES tests and the instructions regarding PHES application were kindly provided to our team by Professor Karin Weissenborn (Hannover - Germany). The copyright for the tests belongs to Hannover Medical School.

To further study the relationship between the PHES results of Romanian cirrhotic patients and the ones from Germany, Korea and Spain we used the normative tables from the three countries. Spain PHES was calculated free online (www.redeh.org). Korean PHES are available in the literature (179). German PHES were offered to our team by Prof. Karin Weissenborn. The results were evaluated comparatively.

Statistics

All the data were collected and added in a database using SPSS (Statistical Package for the Social Science) version 18.0 (Chicago, Illinois, USA). The variables were studied using statistical functions adequate for the expected result. The confidence interval was 95%. A p value less than 0.05 was considered statistically significant.
Chapter XI. Results

The summary of the results has a limited number of figures and tables (with the original numbering from the thesis).

XI.1. PHES standardization for the diagnosis of MHE in northeastern Romania

Two hundred and sixty healthy individuals were included in the PHES validation study. Most of the volunteers were women (53.5%). The median age varied from 19 to 78 years with a mean value of 48.73 ± 14.01 years. The minimal value of the educational level was 4 years and the maximum was 22 years, with a median of 12.37 ± 3.15 years. The socio-demographic characteristics of the study group are detailed in table XI.1.1.I and fig. XI.1.1.4.

Table XI.1.1.I. Mean educational level according to sex and age in the healthy volunteer group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Min</th>
<th>Max</th>
<th>Test F ANOVA p</th>
</tr>
</thead>
<tbody>
<tr>
<td>All group</td>
<td>260</td>
<td>12.37</td>
<td>3.15</td>
<td>0.20</td>
<td>11.99 to 12.76</td>
<td>4</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.450</td>
</tr>
<tr>
<td>Female</td>
<td>139</td>
<td>12.51</td>
<td>3.21</td>
<td>0.27</td>
<td>11.97 to 13.05</td>
<td>4</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>121</td>
<td>12.21</td>
<td>3.08</td>
<td>0.28</td>
<td>11.66 to 12.77</td>
<td>6</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>18-40 y</td>
<td>84</td>
<td>13.00</td>
<td>3.31</td>
<td>0.36</td>
<td>12.28 to 13.72</td>
<td>6</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>41-60 y</td>
<td>105</td>
<td>13.17</td>
<td>2.53</td>
<td>0.25</td>
<td>12.68 to 13.66</td>
<td>8</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>61-80 y</td>
<td>71</td>
<td>10.45</td>
<td>2.98</td>
<td>0.35</td>
<td>9.74 to 11.16</td>
<td>4</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

Figure XI.1.1.4. Mean educational level according to sex and age group in the healthy volunteer group
The results of the neuropsychometric tests in the healthy volunteer group were: DST - 40.3±9.6 points, NCT-A - 41±15.8 seconds, NCT-B - 99.5±40 seconds, SDT - 59.2±10.2 seconds, LTT-t - 87.4±27.7 seconds and LTT-e - 25±15.4 points. Age and education years significantly influenced the final results of the six tests. The DST and SDT median results differed according to sex (p < 0.001).

**PHES validation according to age (PHESa)**

For the 5 psychometric tests (6 subscores), the results were calculated in accordance with the normative tables generated from the median and standard deviations.

The sum of the 6 subscores (LTT was divided in LTT-time and LTT-errors) makes up the final PHESa score. Considering the calculation method the minimum and maximum values of the PHES score can be: -18 (6 * -3 points) and +6 (6 * +1 points).

In the healthy volunteer group the mean PHES value was -0.23 with a standard deviation of 2.077. The cut-off value of PHESa (limit between normal and pathologic scores) was set at -5 points (median minus 2 SD).

**PHES standardization according to age, education and sex**

All of the socio-demographic factors that influenced the psychometric tests in the bivariate analysis were assessed using the multivariate analysis - table XI.1.4.I. The PHES was generated using the 6 formulas resulted from statistical calculations. PHES varied between -6 and +18 points.

**Table XI.1.4.I. Predictors of the psychometric tests - multivariate analysis**

<table>
<thead>
<tr>
<th>Test</th>
<th>r</th>
<th>Rsquare</th>
<th>SD</th>
<th>p</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>DST</td>
<td>0.728</td>
<td>0.530</td>
<td>7.44</td>
<td>0.001</td>
<td>y = 33.84 – 3.95 x gender – 0.30 x age + 1.73 x education</td>
</tr>
<tr>
<td>NCT-A</td>
<td>0.595</td>
<td>0.354</td>
<td>15.98</td>
<td>0.001</td>
<td>y = 41.72 + 0.60 x age - 2.19 x education</td>
</tr>
<tr>
<td>NCT-B</td>
<td>0.650</td>
<td>0.423</td>
<td>38.77</td>
<td>0.001</td>
<td>y = 136.21 + 1.35 x age – 7.53 x education</td>
</tr>
<tr>
<td>SDT</td>
<td>0.462</td>
<td>0.213</td>
<td>11.75</td>
<td>0.001</td>
<td>y = 63.96 + 5.69 x gender + 0.22 x age – 1.22 x education</td>
</tr>
<tr>
<td>LTT-t</td>
<td>0.394</td>
<td>0.155</td>
<td>27.91</td>
<td>0.001</td>
<td>y = 87.78 + 0.61 x age – 2.12 x education</td>
</tr>
<tr>
<td>LTT-e</td>
<td>0.278</td>
<td>0.087</td>
<td>22.45</td>
<td>0.001</td>
<td>y = 20.78 + 0.40 x age – 0.77 x education</td>
</tr>
<tr>
<td>W-LTT</td>
<td>0.394</td>
<td>0.155</td>
<td>19.16</td>
<td>0.001</td>
<td>y = 19.08 + 0.48 x age – 1.20 x education</td>
</tr>
</tbody>
</table>

In the healthy volunteer group the median PHES was 0.43 (min. -3, max. 5) with a standard deviation of 1.37. The cut-off value for PHES was set at -3. Seven out of 260 patients (2.6%) were outside the limits (> -3).

**XI.2. Evaluation of the neuropsychometric tests in the liver cirrhosis group.**

Among 106 patients with cirrhosis, 50.9% were men and the median age of the group was 55.6 ± 11.2 years. The years of education varied between 4 and 17, with a median value of 11.3 ± 3 years. The complex socio-demographic, clinical and biological data of the cirrhosis group are presented in table XI.2.1.VI.
Table XI.2.1.VI. Demographic, clinical and biological characteristics of the patients with liver cirrhosis

<table>
<thead>
<tr>
<th>Demographic, clinical and laboratory data</th>
<th>Cirrhotic patients (N = 106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.6 ± 11.2 (22-78)</td>
</tr>
<tr>
<td>Education (years)</td>
<td>11.3 ± 3 (4-17)</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>52 (49.1%)/54 (50.9%)</td>
</tr>
<tr>
<td>Etiology of cirrhosis (alcoholic/virus C/virus B/ autoimmune)</td>
<td>40(37.7%)/38(35.8%)/25(23.6%)/3(2.8%)</td>
</tr>
<tr>
<td>MELD score</td>
<td>12.3 ± 5.16 (6-28)</td>
</tr>
<tr>
<td>Child-Pugh class (A/B/C)</td>
<td>46 (43.4%)/43(40.6%)/16(17%)</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>2.6 ± 2.8 (0.4-15.6)</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>3.6 ± 0.7 (2.4-5.1)</td>
</tr>
<tr>
<td>INR</td>
<td>1.3 ± 0.3 (0.9-2.5)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.8 ± 0.3 (0.4-2.2)</td>
</tr>
<tr>
<td>Na (mmol/l)</td>
<td>136.9 ± 3.5 (126-143)</td>
</tr>
<tr>
<td>K (mmol/l)</td>
<td>4.2 ± 0.5 (2.9-5.9)</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.9 ± 1.3 (0.01-6.8)</td>
</tr>
<tr>
<td>Venous ammonia (µg/dl)</td>
<td>81.5 ± 34.2 (29-199)</td>
</tr>
</tbody>
</table>

In cirrhotics, the results of the 6 neuropsychometric tests (DST, NCT-A, NCT-B, SDT, LTT-t and LTT-e) were: 29.5±9.6 points, 56.6 ± 23.9 seconds, 149.1 ± 57.1 seconds, 72 ± 15 seconds, 104.1 ± 33 seconds and 48.8 ± 29.9 points. In the liver cirrhosis group DST, NCT-A, NCT-B, SDT and LTT-t were significantly correlated with the number of years of education. (p < 0.05). Only NCT-A and NCT-B were associated with age (p < 0.05).

All of the test from the cirrhosis group showed inferior median values compared to the ones from the healthy volunteers (p<0.001) - fig. XI.2.3.1. These results underline the high proportion of cirrhotic patients with neurologic impairment.

![Figure XI.2.3.1. Median scores of the neuropsychometric tests in cirrhotics and healthy volunteers](image-url)
XI.3. PHES characteristics in patients with cirrhosis

In cirrhotic patients PHES varied between -13 and 4 points with a median value of -2.44±3.40. Comparatively (healthy vs. cirrhotics) the median PHES was significantly lower in the chronic liver disease group (-2.44 vs. 0.43; p=0.001). These results are similar to those observed when the individual psychometric tests were compared.

PHES was directly correlated with age in cirrhotic patients (r= +0.188; p=0.05). Active drinkers had a lower PHES than non-drinkers (-3.77 vs. -1.89, p = 0.009). Sex and educational level showed no correlations with PHES (p<0.05).

In the liver cirrhosis group there was a direct correlation between PHES and serum bilirubin, Sodium, albumin, INR and C reactive protein (p = 0.001). There were no associations between PHES and serum Potassium, creatinine, alpha-fetoprotein or ammonia (p > 0.05). Approximately 53% of patients showed a low PHES associated with high values of the Child-Pugh and MELD scores (p = 0.001).

For the PHES cut-off (-3 points), MHE was diagnosed in 37 cirrhotic patients (34.7%). PHESa showed a median value of -5.25±4.2. According to the lower limit of PHESa (-5), 37.7% of patients had MHE.

PHESa was significantly correlated with PHES in Romanian cirrhotic patients (p = 0.001, r = +0.857). The strong correlation between the two scores confirms the validity of the standardization process.

As shown in table XI.3.3.1., serum Sodium, INR, albumin, bilirubin and C reactive protein had significantly different median values in patients with or without MHE (p < 0.05). The liver function (evaluated using Child-Pugh and MELD scores) was significantly lower in patients with MHE compared to those without MHE.

Table XI.3.3.1. Median values of laboratory parameters in patients with/without MHE

<table>
<thead>
<tr>
<th>Laboratory parameter</th>
<th>PHES≤-3 (n=37)</th>
<th>PHES&gt;-3 (n=69)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na, mmol/l</td>
<td>135.86±3.65</td>
<td>137.59±3.30</td>
<td>0.015</td>
</tr>
<tr>
<td>K, mmol/l</td>
<td>4.32±0.73</td>
<td>4.27±0.47</td>
<td>0.669</td>
</tr>
<tr>
<td>INR</td>
<td>1.48±0.42</td>
<td>1.18±0.18</td>
<td>0.001</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>3.29±0.66</td>
<td>3.84±0.71</td>
<td>0.001</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
<td>34.46±20.01</td>
<td>33.88±15.63</td>
<td>0.870</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.89±0.30</td>
<td>0.83±0.27</td>
<td>0.304</td>
</tr>
<tr>
<td>Total bilirubin, mg/dl</td>
<td>4.11±4.10</td>
<td>1.79±1.14</td>
<td>0.001</td>
</tr>
<tr>
<td>Conjugated bilirubin, mg/dl</td>
<td>2.58±2.68</td>
<td>0.90±0.66</td>
<td>0.001</td>
</tr>
<tr>
<td>PCR, mg/dl</td>
<td>1.58±0.30</td>
<td>0.65±0.10</td>
<td>0.001</td>
</tr>
<tr>
<td>AFP, UI</td>
<td>10.35±2.21</td>
<td>11.59±1.75</td>
<td>0.667</td>
</tr>
<tr>
<td>Ammonia, µg/dl</td>
<td>82.78±39.83</td>
<td>80.93±31.16</td>
<td>0.792</td>
</tr>
</tbody>
</table>

XI.4. Comparative evaluation of the study PHES with PHES from other countries

Study PHES versus PHES Germany in healthy volunteers

In order to adequately compare the test results from Romania and Germany we used PHESa. The PHESa results of the healthy volunteers from the two countries were evaluated using the normative tables.

On the German normative data, the PHESa score from Romania was significantly lower from that of Germany (-2.33 vs. 0.48; p < 0.001). Also, the standard deviation showed higher values (2.760 vs. 1.940; p < 0.001).
Using the Romanian normative data, PHESa Germany had a higher median value compared to the PHESa from Romania (1.96 vs. -0.23, p < 0.001). There were no significant differences in SD.

Correlations of the study PHES with PHES Korea, PHES Spain and PHES Germany in cirrhotic patients

To correlate the cirrhotic PHES values from our study with the ones from other countries we used the PHES standardized according to age, education and sex. The results of the neuropsychometric tests from the Romanian study were interpreted according to the normative data in Spain, Korea and Germany.

The results showed significant statistic correlations between the Romanian study PHES and the PHES from Spain (A), Korea (B) and Germany (C) - fig. XI.4.2. The tightest association was with PHES Spain (r = +0.941).

Figure XI.4.2. Correlations between PHES Romania and PHES Spain, PHES Korea and PHES Germany for the patients with liver cirrhosis
Chapter XII. Discussions

PHES is a first-line test for the diagnosis of MHE according to ISHEN and Viena Consensus recommendations (35,163). The psychometric tests are inexpensive, with high sensitivity and specificity for MHE diagnosis and easy to apply by the clinician/investigator (181). However, PHES is not the ideal test and has some disadvantages. PHES cannot be applied to illiterat patients. In our study we used illiteracy as an exclusion criteria in order to prevent potential interpretation errors. To avoid this inconvenience the NCT-B test could be replaced with the Figure-connection test that uses figures and drawings. This test was applied by Dhiman et al. in the Indian population instead of NCT-B because of the high proportion of illiterat patients (178). PHES has a learning effect. Studies show that PHES results in cirrhotic patients are significantly higher on a second run (after different periods of time), without treatment. The NCT-A and NCT-B tests showed the highest learning effect (175,347). For this reason 4 different types of PHES are available for investigators and clinicians. Ennen and Goldbecker showed the absence of the learning effect when using different types of PHES for re-testing (184,185).

The time needed to complete the test is a major disadvantage for this evaluation. Previous studies showed that the median time for completion is 20 minutes(170). In our study one of the investigators reported a maximum time of 45 minutes in a patient with liver cirrhosis and MHE. Further studies are needed in order to evaluate the median time to complete the PHES with respect to the different socio-demographic characteristics of each country. Moreover, new studies could evaluate the possibility of using fewer test for MHE diagnosis.

In our study the liver cirrhosis group was not stratified according to age, education and severity of liver disease. Furthermore, the number of cirrhotic patients was significantly lower than the number of healthy individuals (106 vs. 260). This is due to very restrictive inclusion criteria used for cirrhotic patients in order to reduce the factors that influence the test results. Also, the cirrhosis group was only used to comparatively assess the results of the PHES with the ones of the healthy volunters and to estimate the prevalence of MHE.

The healthy subject group is not a numerically representative sample for the population of Romania. Moreover, only patients from a single tertiary care center were included in the group. However, the majority of the Romanian citizens are caucasian and the socio-demographic features are similar in all geographical areas of our country. Therefore, we can consider the healthy volunteer group to be representative for the Romanian population.

The PHES calculation methodology varies among previous studies (174-183). In our research we used a statistical procedure commonly used in the PHES studies. The psychometric test results were rounded to integer values considering the SD interval. In 2008, Amodio et al proposed a new method for PHES calculation using the Mean Psychometric Z Score (MZPS). This variable represented the mean Z score derived from statistical calculations. Approximated variables have a lower precision than averaged variables. Based on these facts we can assume that the standard PHES determination is inferior compared to the one that uses MZPS. Nonetheless, the italian researchers failed to show significant differences regarding the ability of the two scores to diagnose MHE. In 100 cirrhotic patients both scores revealed the same 25 patients with MHE (175). Considering the literature data we can conclude that further studies are necessary to prove the superiority of the MZPS standardization method over the standard validation method.

There are scientific controversies regarding the interpretation of LTT and PHES. LTT can be considered a single score (w-LTT or LTT-sum) or divided in two separate scores: LTT-t and LTT-e. The original score promoted by Hamster and Schomerus had two separate results. The two scientists proved that the number of errors and the time needed to complete
the test are independent factors in the discriminative analysis between healthy subjects and cirrhotic patients. Moreover, precision and motor speed are two separate neurologic functions that can be independently altered in various neuropsychiatric conditions. Therefore, both tests were included in the final PHES calculations (165). Considering these facts, in the present study we used both LTT-t and LTT-e to compute the PHES. However, the Italian researchers analyzed the two tests and confirmed that they are tightly related (175). These conclusions were further confirmed in the Polish PHES standardization study (181). Amodio et al. proposed a simplified single score (w-LTT) in order to facilitate the statistical calculations and to enhance the accuracy of the test (175). Seo et al. comparatively evaluated the accuracy of the two tests in determining MHE and showed no differences (179). W-LTT might be superior to LTT-t and LTT-e when determining the PHES.

In the previous standardization studies EHM prevalence varied between 22% and 49% (174-183). The different study protocols and heterogeneous populations are possible explanations for these variations. In our study the distribution of the patients was homogeneous considering the socio-demographic factors and the severity of the liver disease.

The highest MHE prevalence was reported in China PHES study - 49%. These results must be interpreted with caution because the majority of patients with cirrhosis were Child-Pugh B and C. Moreover, the mean number of education years was the lowest from the PHES standardization studies: 8.2 ± 3.6 years (182). Similar data were reported in India where MHE prevalence was 48% (178).

In Korea, 160 patients with liver cirrhosis without HE were evaluated using PHES. Contrary to China and India, the mean age was high (10.7 ± 3.9 years) and most of the patients were included in Child-Pugh A (80.6%). Only 41 patients had MHE (25.6%) (179). Similar data were reported in Taiwan where 85.1% of patients with cirrhosis were Child-Pugh A. EHM was proved in 28.7% of the subjects (183).

In Poland, Wunsch et al. applied the PHES on a group of 71 patients with cirrhosis without clinical signs of HE. Most of the patients were included in Child-Pugh A (74%). Also, the mean number of education years was the highest among the PHES standardization studies (13.1 ± 3.4 years). Only 22% of the patients had MHE (181).

In our study the cirrhotic patients were homogeneously distributed according to the severity of the liver disease: 43.4 % - Child-Pugh A and 56.6% - Child-Pugh B and C. The median number of education years was 11.37 ± 3.0 without significant variations regarding sex and age. Considering these data our group can be considered representative for the Romanian cirrhotic population.

We can conclude that PHES determined MHE prevalence in Romania (34.7%) has a mean value compared to previous studies (22-49%) (174-183). The future studies should use homogeneous study groups in order to accurately determine the prevalence of MHE.

In Germany, Korea and India, PHES was standardized only according to age (174,178,179). Weissenborn et al. found no significant differences between the two PHES standardization methods: dependent on age or dependent on all the socio-demographic factors that influence the psychometric tests (170).

In our study, PHESa had a median value of -5.25 in the cirrhotic group. According to the cut-off level (-5), 38.6 % of the patients were diagnosed with MHE (n=41). The use of this method showed a higher prevalence of MHE compared to PHES (38.6% vs. 34.7%). The small difference (4 patients) is due to the influence of the demographic factors (education years and sex) included in the statistical calculations. Considering the high influence of these additional factors on the individual psychometric tests, PHES can be considered superior to PHESa in Romania. Another argument to support this statement is the multivariate PHES analysis that showed the number of education years as an independent factor for the PHES results.
The difference between the two tests (PHES and PHESa) in estimating MHE prevalence in Romania, was not statistically significant (p > 0.05). The Korean standardization study showed similar results. Seo et al. evaluated PHES and PHESa for MHE diagnosis on 160 patients with liver cirrhosis. The estimated prevalence for the two standardization method was: 25.6% - PHESa and 27.5% - PHES. The differences between the two results were not statistically significant (p > 0.05) (179).

In the Korean study there were also no differences between the median values of PHES and PHESa in the healthy volunteer group (-0.31 vs. -0.25) (p > 0.05) (179). In our healthy study group we showed similar results: 0.43 - PHES vs. -0.23 PHESa (p > 0.05).

In the same Korean study the authors demonstrated significant correlations between PHES and PHESv (90.6% of the patients with cirrhosis had similar results) (179). In our study PHES was directly and significantly correlated with PHESa (85.7% of the PHESa results correlated with the PHES results).

These observations underline the validity and precision of the Romanian PHES standardization study. There were no significant differences between the two PHES validation methods. We consider the multifactorial PHES standardization superior to PHESa because its calculation involves the education (a significant factor for PHES determination in Romania). However, due to the lack of statistical differences between the two standardization methods both methods can be used to interpret the psychometric test results and diagnose MHE in Romania.
Chapter XIV. Conclusions

1. The epidemiologic characteristics of the Romanian healthy individuals were similar to those published in the previous PHES standardization studies. Age and sex distribution was homogeneous. Most of the subjects were from urban areas. The educational level had an uniform distribution and was inversely correlated with age. Half of the healthy subjects were active alcohol drinkers. The healthy volunteer group can be considered representative for the Romanian population.

2. In the healthy group, the results of the 5 neuropsychometric tests - 6 scores (DST, NCT-A, NCT-B, SDT, LTT-t and LTT-e) were significantly influenced by the educational level. DST and SDT were different in males compared to females. We can conclude that the socio-demographic are significant determinants in the interpretation of the psychometric tests.

3. There was a strong correlation between LTT-e and LTT-t that suggests an association between the dominant motor functions of the brain (speed - accuracy). The results are similar to previous standardization studies.

4. PHES was standardized according to age (PHESa) for MHE diagnosis in a population from northeastern. Based on the results of the psychometric tests and on the linear regression scales, normative tables for Romanian PHESa were generated. The cut-off between normal and pathological values was set at -5 points.

5. PHES was validated according to age, education and sex. The cut-off value was set at -3 points. The results are numerically superior to other literature studies. These differences can be due to the socio-demographic characteristics of the Romanian population.

6. The number of education years was the only factor that significantly influenced the final PHES score results of the healthy volunteers. The results are similar to previous studies and confirm the need of PHES standardization in a population before applying it for MHE diagnosis.

7. The liver cirrhosis group had a homogeneous distribution with respect to sex and age. The majority of the cirrhotics were not active alcohol drinkers.

8. The leading cause of cirrhosis in our group was ethanol consumption followed by hepatic virus C and B infections. Most of the patients were included in Child-Pugh B and C classes. The median MELD score was 12,3. The Child-Pugh and MELD scores were higher in young people and in patients with a history of chronic alcohol consumption.

9. In the liver cirrhosis group the educational level was the only variable that influenced the results of the neuropsychometric tests.

10. The six psychometric scores had lower median values in cirrhotics compared to healthy volunteers. Similar data were shown in previous PHES standardization studies.

11. Serum Sodium, albumin, bilirubin, INR and C reactive protein levels correlated with the PHES score. The liver disease severity scores (Child-Pugh and MELD) were significantly correlated with the PHES results. We can conclude that an advanced liver disease is more likely to be associated with cognitive alterations.
12. MHE prevalence in the liver cirrhosis group was 34.7%. Our result is consistent with previous published values (20-80%).

13. The laboratory parameters (Sodium, albumin, bilirubin, INR and C reactive protein levels) and the liver disease severity scores were significantly lower in MHE patients compared to non-MHE patients.

14. The Romanian PHES normative tables will generate superior results of the PHES score compared to the German normative tables. These results should be interpreted in light of the different socio-demographic characteristic between the two countries.

15. The Romanian cirrhotic PHES values were correlated with the ones interpreted on the normative tables from Spain, Korea and Germany. These results confirm the validity of the Romanian PHES standardization study.

**Keywords:** minimal hepatic encephalopathy, psychometric hepatic encephalopathy score, neuropsychometric test, liver cirrhosis
Selected references


