Minimal Hepatic Encephalopathy: Standardization of definition and diagnosis; Clinical Signification and Prognosis

“Gr.T. Popa” University of Medicine and Pharmacy

PhD THESIS
SUMMARY

Minimal Hepatic Encephalopathy: Standardization of definition and diagnosis; Clinical Significance and Prognosis

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Minimal Hepatic Encephalopathy: Standardization of definition and diagnosis; Clinical Signification and Prognosis

Thanks to

Professor Anca Trifan
Professor Carol Stanciu
My family
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INTRODUCTION

Hepatic encephalopathy (HE) is a serious neuropsychiatric complication of both acute and chronic liver failure, with a significant impact on the quality of life and a predictive value of poor outcome. Although the origin of the toxins responsible for the altered mental has not been yet completely elucidated, the role of ammonia remains central, supported by a large number of studies (1,2). In cirrhosis, the degree of liver failure and the presence of portosystemic shunts are responsible for increased levels of serum ammonia. However hyperammonemia is difficult to evaluate in the context of HE, given the fact that correlations between ammonia levels and the severity of HE have proved to be extremely variable (3).

The annual risk of developing overt HE in cirrhotic patients is 20%. An estimated one-third to one-half of hospitalizations for cirrhosis are related to overt HE, and the frequency of hospitalization for overt HE has nearly doubled over the last decade.

When cirrhotic patients are found normal under neurological clinical examination, but have mild cognitive and psychomotor deficits, the condition is referred to as minimal hepatic encephalopathy (MHE).
CHAPTER VII
MOTIVATION AND OBJECTIVES

Reaching a diagnosis of MHE can be difficult due to the lack of a gold standard test. Diagnosis of MHE is still controversial, especially when it comes to establish criteria and reliable diagnostic tests easily applied in clinical practice. Neurological and psychological evaluations do not offer sufficient clinical tools for diagnosing MHE and assessing its progression to overt HE. Current methods for MHE diagnosis are subjective, time-consuming, costly and hard to access. This might explain why most clinicians do not recommend testing for MHE in current clinical practice.

Moreover, while it is generally accepted that ammonia plays a central role in the pathophysiology of hepatic encephalopathy (4), the mechanisms of its neurotoxicity remain poorly understood.

Oxidative stress, a condition arising from an imbalance between toxic reactive oxygen species (ROS) and antioxidant systems has been reported to be implicated in the development and the progression of various pathological conditions (5), including acute and chronic liver diseases. In this way, a previous number of studies have focused on the role of oxidative stress in liver injury (6-10). Additionally, an increasing number of studies in various animal models (11-15) have described a possible relevance of oxidative stress as a pathogenetic mechanism involved in HE (16). Still, there are very few studies regarding these aspects in human patients, only related to alcohol poisoning-induced oxidative stress status in HE (17).

Considering the high prevalence of MHE, there are several controversies related to the testing and treatment of
this condition. Is it a medical error if we do not screen cirrhotic patients for MHE?\(^{(18)}\) Is it better to treat all cirrhotic patients without testing MHE? There is no current consensus on how MHE should be treated. The decision to initiate therapy is difficult since MHE is asymptomatic or causes few symptoms. As a result, the expected adherence to treatment is poor. Due to uncertainties about the therapy, future studies should identify the risks and efficacy of long-term treatment, to be implemented in current practice.

The development of overt HE is considered a sign of a poor prognosis and patients should receive therapy for an indefinite period of time or until they undergo liver transplantation. Therefore prevention of overt HE is warranted. The term secondary prophylaxis was proposed to define the therapy administered to prevent recurrence of overt HE in patients with a history of overt HE episodes. Currently, the options for a long-term treatment of HE are lactulose, a nonabsorbable disaccharide, and rifaximin, a minimally absorbed oral antibiotic. Several studies have demonstrated the efficacy of rifaximin and lactulose for the maintenance of remission from HE and decrease in hospitalization requirement\(^{(19)}\).

The lack of data regarding MHE in romanian population vindicate once again the theme of the present thesis.

**MAIN OBJECTIVES**

- to evaluate the prevalence of MHE and the risk of this condition for overt HE in cirrhotic patients
● to evaluate several oxidative stress markers (two antioxidant enzymes: superoxide dismutase-SOD and glutathione peroxidase-GPX, as well as a lipid peroxidation marker: malondialdehyde-MDA) in cirrhosis with or without HE

● to evaluate the role of oral glutamine challenge (OGC) and ammonemia in improving the diagnostic performance for MHE

● to develop an algorithm for testing patients for MHE

● evaluation of MHE in patients treated for the maintenance of remission from overt HE

SECONDARY OBJECTIVES

● to evaluate the incidence and predictive factors in overt HE in cirrhosis

● to establish the correlations between the severity of liver disease according to Child-Turcotte-Pugh and MELD scores and levels of the aforementioned oxidative stress markers, as well as with ammonia level

● to evaluate the efficacy of rifaximin versus lactulose for reducing the recurrence of overt HE in cirrhosis (secondary prophylaxis of HE in cirrhosis)
CHAPTER VIII
PATIENTS AND METHODS

Patients
The study included 230 patients diagnosed with liver cirrhosis, hospitalized or followed-up in an outpatient clinic at the Gastroenterology and Hepatology Institute, “St. Spiridon” University Hospital, Iasi, Romania, between March 2010 and December 2012.

General inclusion criteria were: age > 18 years, confirmed diagnosis of cirrhosis, different grades of liver failure (Child, MELD)

General exclusion criteria were: inability to perform psychometric tests, use of antibiotics, sedatives or lactulose in the previous 3 months, recent (< 6 months) active alcoholism, abnormal value for alpha-fetoprotein, diabetes mellitus, neurologic or psychiatric disorders, evidence of decompensated respiratory, cardiac and renal disease.

Diagnostic methods in hepatic encephalopathy
Grading of the symptoms of HE was performed according to West Haven classification system (20):

- Grade 0 - lack of detectable changes in personality or behavior;
Grade 1 - trivial lack of awareness; shortened attention span; impaired addition or subtraction; hypersomnia, insomnia, or inversion of sleep pattern; euphoria, depression, or irritability; mild confusion; slowing of ability to perform mental tasks

Grade 2 - lethargy or apathy; disorientation; inappropriate behavior; slurred speech; obvious asterixis; drowsiness, lethargy, gross deficits in ability to perform mental tasks, obvious personality changes, inappropriate behavior, and intermittent disorientation, usually regarding time

Grade 3 - somnolent but can be aroused; unable to perform mental tasks; disorientation about time and place; marked confusion; amnesia; occasional fits of rage; present but incomprehensible speech

Grade 4 - coma with or without response to painful stimuli

Patients with normal mental status were tested for MHE using Psychometric Hepatic Encephalopathy Score (PHES), a standardized battery of neuropsychological tests including NCT-A (number connection test-A), NCT-B (number connection test-B), DST (digit symbol test), SDT (serial-dotting test) and LTT (line-tracing test). The results of PHES were determined using an online free calculator, available at http://www.redeh.org/TEST_phes.htm, based on the normality tables in the Spanish population. The diagnosis of MHE was defined by a PHES value ≤ -5.

Ethical considerations

The study was conducted according to the provisions of the Helsinki Declaration and was approved by the local Ethics
Committee. All subjects gave their written informed consent.

**Data analysis**

Statistical analysis was performed using Medcalc 12.3.0 and Microsoft Office Package. Both descriptive and inferential statistic methods were used.

CHAPTER IX
RELEVANCE OF OXIDATIVE STRESS IN LIVER CIRRHOSIS WITH OR WITHOUT HEPATIC ENCEPHALOPATHY

The prospective study included 78 patients aged between 41 to 75 years (average 56.3 ± 1.53 years), 52 males and 26 females, with a definite diagnosis of cirrhosis, in a stable condition, which were recruited from the outpatient clinic or hospitalized between January 2012 and August 2012. The control group consisted of 19 healthy subjects matched to patients according to age, sex, and education level.

Subjects with gastrointestinal bleed or blood transfusion within previous 2 weeks, infections, smoking, taking antioxidant supplements were excluded. The ongoing medication was not suspended before the tests since the lack of the treatment in cirrhotic patients was considered unethical.

Of the 78 patients, 12 were diagnosed with overt HE, 24 had MHE and 42 had neither overt HE nor MHE (fig 1).
Analysis of covariance showed that patients from all 3 groups did not differ significantly from healthy subjects, with respect to age (p = 0.803, F= 0.22) and gender (p = 0.867, F= 0.14).

The etiology of cirrhosis was alcohol in 34 patients, chronic viral hepatitis in 36 patients, while both alcohol and viral factor were found in 8 cases (fig 2).

Patients had different grades of liver failure according to Child classification (A-C) (fig 3) and MELD score (10-25).
Figure 3. Severity of liver disease according to Child score.

METHODS

- **Determination of GPX** - dithio-bis-dinitro-benzoic acid (DTNB) method
- **Determination of SOD** - SOD Assay Kit (FLUKA, 19160)
- **Determination of MDA** - thiobarbituric acid reactive substances (TBARs) assay

RESULTS

Regarding the specific activity of SOD, it was observed a significant difference between the groups (F(3,93)=11, p<0.0001). Post-hoc comparisons also showed a significant increase in SOD activity in overt HE group (p<0.0001) vs controls, as well as in MHE group (p<0.0001) and non-HE group (p<0.0001), when compared to controls. No significant differences were found between overt HE vs MHE (p=0.303), HE vs non-HE (p=0.818) and MHE vs non-HE group(p=0.088) (fig 4).
Figure 4. Superoxide dismutase specific activity in the serum. ***p < 0.0001 vs control group.

We also noted a significant differences in the GPX specific activity in the serum between our groups (F(3.93)=7, p=0.00013). Moreover, when we performed the post-hoc analysis, we observed a significant decrease of GPX specific activity in overt HE (p=0.003), MHE (p=0.002) and also non-HE group (p=0.003), as compared to the controls. Also, a significant decrease of GPX was found in overt HE group, as compared to non-HE group (p=0.027), but no significant differences between overt HE vs MHE (p=0.161) and MHE vs non-HE groups (p=0.274) (fig 5) were found.
Figure 5. Glutathione peroxidase specific activity in the serum. **p < 0.003 vs control group.

Regarding MDA concentration, we observed very significant differences between our groups (F(3.93)=47, p<0.0001). Additionally, post-hoc analysis showed a significant increase of MDA in overt HE group (p<0.0001), MHE group (p<0.0001), as well as in non-HE group (p<0.0001) when compared to the controls. A significant increase in MDA concentration was also seen in overt HE group, as compared to MHE group (p<0.0001) or non-HE group (p<0.0001) suggesting that MDA could be a physiopathological marker for HE (fig 6).
Figure 6. Malondialdehyde concentration in the serum. ***p < 0.0001 vs control group.

Activity of the oxidative stress markers is presented comparatively in the table I.

<table>
<thead>
<tr>
<th>SOD (U/ml)</th>
<th>GPX (U/ml)</th>
<th>MDA (nM/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.542 ± 0.05</td>
<td>0.091 ± 0.01</td>
</tr>
<tr>
<td>Overt EH</td>
<td>1.186 ± 0.13</td>
<td>0.04 ± 0.006</td>
</tr>
<tr>
<td>MHE</td>
<td>1.011 ± 0.18</td>
<td>0.054 ± 0.008</td>
</tr>
<tr>
<td>non-EH</td>
<td>1.222 ± 0.14</td>
<td>0.061 ± 0.01</td>
</tr>
</tbody>
</table>

When we analyzed the correlations between the oxidative stress markers and the levels of venous ammonia, we observed significant correlations between GPX vs venous blood ammonia (n=78, r= -0.233, p= 0.043)(fig 7) and for MDA vs venous blood ammonia (n=78, r= 0.519, p< 0.0001)(fig 8), but not for SOD vs venous blood ammonia (n=78, r= -0.045, p= 0.696).
Similarly, we found significant correlations between the oxidative stress markers and MELD and Child scores for GPX: GPX vsMELD \((n=78, r= -0.331, p=0.003)/GPX vs Child \((n=78, r= -0.230, p= 0.048)\) (fig 9) and MDA: MDA vs MELD \((n=78, r= 0.428, p< 0.0001)/MDA vs Child \((n=78, r= 0.324, p= 0.004)\) (fig 10), but not for SOD:SOD vs MELD \((n=78, r= -0.061, p= 0.593)/SOD vs Child \((n=78, r= -0.173, p= 0.13)\).
Figure 9. Correlations GPX vs CHILD (n=78, r= -0.230, p= 0.048), respectively
GPX vs MELD (n=78, r= -0.331, p= 0.003).

Figure 10. Correlations MDA vs CHILD (n=78, r= 0.324, p= 0.004), respectively
MDA vs MELD (n=78, r= 0.428, p< 0.0001).
CHAPTER X
SIGNIFICANCE OF ORAL GLUTAMINE
CHALLENGE FOR THE DIAGNOSIS OF MINIMAL
HEPATIC ENCEPHALOPATHY IN CIRRHOSIS

This prospective study included 54 patients diagnosed with liver cirrhosis, hospitalized or followed-up in an outpatient clinic at the Gastroenterology and Hepatology Institute, “St. Spiridon” University Hospital, Iasi, Romania, between March 2010 and June 2011.

Additional inclusion criteria were: normal neurologic signs upon examination, grade 0 of HE (West-Haven criteria), absence of previous HE episodes.

Additional exclusion criteria were: overt HE (grade 1 or higher), gastrointestinal bleed, infection, spontaneous bacterial peritonitis within previous 3 months.

The control group consisted of 16 healthy subjects matched to patients according to age, sex, and education level.

Analysis of covariance showed that subjects from the study group did not differ significantly from controls, with respect to age, gender and level of education (tab II).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study group (n=54)</th>
<th>Controls (n=16)</th>
<th>F*</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>59.5 ± 4.2</td>
<td>53.37 ± 5.4</td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td>Sex (M / F)</td>
<td>34 / 20</td>
<td>11 / 5</td>
<td>0.17</td>
<td>0.67</td>
</tr>
<tr>
<td>Education: elementary/high school/ university</td>
<td>14 / 27 / 13</td>
<td>3 / 8 / 5</td>
<td>0.5</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Most of the patients had toxic cirrhosis (62.96%). 50 (92.6%) of 54 patients had mild and moderate liver failure.
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According to Child and MELD scoring.

METHODS

Arterial ammonia blood level assessment, together with the psychometric tests were performed pre- and post-glutamine load.

In a fasting state, patients and healthy control subjects ingested a 20g solution of glutamine dissolved in 100 ml tap water. Any symptoms that appeared during one hour were recorded. Samples of arterial blood (radial puncture) were taken at baseline and 60 minutes after glutamine load. Each patient and healthy subject performed the PHES at baseline and at 60 minutes after OGC.

Statistical analysis was performed using Medcalc 12.3.0 and Microsoft Office Package. The results were expressed as means ± SD. We used the receiver operating characteristics (ROC) curve in order to analyze the sensibility and the specificity of blood ammonia level (pre- and post-glutamine) as a tool for the diagnosis of MHE and for establishing optimal threshold values. Correlations between variables were examined with a Pearson correlation. Multivariate regression was performed for the determination of individual predictive factors for overt HE.

RESULTS

Ammonia blood levels
Post-glutamine load, a significant raise of arterial ammonia levels in patients with cirrhosis (85.2±20.8 versus 159.82±66.01 μg/dL, p <0.0001) was observed, while in healthy control subjects the changes did not reach the level of
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Using the ROC curve analysis, cut-off values of ammonia blood levels both at baseline and post-OGC were defined (87.8 μg/dL and 124μg/dL, respectively). For the diagnosis of MHE, baseline blood ammonia showed an area under the ROC curve (AUROC) of 0.54 (CI: 0.402-0.680, p= 0.58), while the post-OGC was 0.53 (CI: 0.389-0.667, p=0.77) (fig 11 A,B).

Figure 11. ROC curves showing the sensitivity and specificity of baseline (A) and post- glutamine load (B) arterial blood ammonia levels for the diagnosis of MHE.
Prevalence of MHE before and after OGC

At baseline, 29 of 54 patients (53.7 %) met the PHES criteria for MHE diagnosis. After glutamine load, the percentage of patients diagnosed with MHE increased to 43 (79.6 %). The values of PHES were significantly lower post-OGC compared to baseline (P<0.0001), suggesting that OGC increased the diagnostic performance of PHES for MHE in cirrhotic patients, and it remained almost unchanged in healthy subjects (fig 12).

An altered OGC defined by ROC curve as an increase of ammonia over 124 μg/dL post-glutamine load was found in 37 patients (68.51%). Among these, 30 (81.1%) had MHE, while 7 (18.9%) did not have MHE.

Incidence of overt HE in the follow-up and predictive factors

On follow-up, 10 patients (18.51%) developed overt HE. Among these, 9 had MHE (4 at baseline and 5 after glutamine
load), and 1 patient met no criteria for MHE both at baseline and post-glutamine.

The development of overt HE was significantly associated with the post-glutamine PHES score (n= 54, r= -0.382, p= 0.004), while PHES alone did not show any significant correlation (n= 54, r= -0.140, p= 0.313).

The following variables were considered in a predictive model for overt HE: Child and MELD scores, grade of esophageal varices, pre- and post-glutamine arterial blood ammonia. In the multivariate regression analysis MELD score (OR = 1.5187, 95% CI: 1.0690 – 2.1574, p = 0.0197) and MHE (OR= 2.0402; 95% CI, 0.9457 - 4.4015, p= 0.0288). were independent predictive factors for the development of overt HE.

CHAPTER XI
EVALUATION OF MINIMAL HEPATIC ENCEPHALOPATHY IN PATIENTS TREATED FOR THE MAINTENANCE OF REMISSION FROM OVERT HEPATIC ENCEPHALOPATHY. EFFICACY OF RIFAXIMIN VERSUS LACTULOSE FOR REDUCING THE RECURRENCE OF OVERT HEPATIC ENCEPHALOPATHY IN CIRRHOSIS

This prospective study included 98 patients diagnosed with liver cirrhosis, hospitalized or followed-up in an outpatient clinic at the Gastroenterology and Hepatology Institute, “St. Spiridon” University Hospital, Iasi, Romania, between October 2010 and December 2011.

Adititional inclusion criteria were: patients with at least one episode of overt HE in the history (grade 2 or more according
to West-Haven criteria) and remission at baseline (grade 0 or 1 according to West-Haven criteria).

Additional exclusion criteria were: gastrointestinal bleed, spontaneous bacterial peritonitis, transjugular intrahepatic portosystemic shunt (TIPS) within previous 3 months, anemia (Hb<8g/dL), hyponatremia (Na<125mmol/l).

METHODS

Patients who recovered from HE were grouped according to one of the following therapies:

- RI- group (38 patients) - rifaximin intermittently, 400 mg three times daily, 14 days/month
- RD-group (28 patients) - rifaximin 400 mg three times daily
- L-group (32 patients) - lactulose 30 to 60 mL in 2 or 3 divided doses daily

for a 6-month period or until the development of the first episode of HE (grade 2 or more according to West-Haven criteria). The follow-up lasted 12 months.

RESULTS

At baseline, prevalence of MHE was 55.1%, with no significant differences between the groups (p=0.641). After 3 months, we noted the maintenance of remission from MHE in 64.81% out of all patients, with no significant differences between the groups (p=0.969)(fig 13). During follow-up, at 6 months and 12 months, the percentage of patients without MHE decreased to 44.44%, and 38.88%, respectively.
Of the 98 patients, overt HE episodes occurred in 24 (24.48%) during the 12-month study period. Probability of developing recurrent overt HE, illustrated by a Kaplan-Meier analysis, was not significantly different in the three groups: 26.31% in RI group, 25% in RD group versus 21.87% in L group (p=0.448) (fig 14).

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**Figure 13.** Evaluation of MHE in patients treated for the maintenance of remission from overt HE.

**Figure 14.** Probability of developing recurrent overt HE in the three groups (p=0.448).
We observed significant correlations between recurrent overt HE and MELD score (n=98, r= 0.343, p= 0.001) and PHES (n=98, r= -0.224, p= 0.026) (table III).

Table III. Correlations between recurrent overt HE and baseline parameters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pearson’s coefficient (r)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child</td>
<td>0.177</td>
<td>0.082</td>
</tr>
<tr>
<td>MELD</td>
<td><strong>0.343</strong></td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>PHES</td>
<td><strong>-0.224</strong></td>
<td><strong>0.026</strong></td>
</tr>
<tr>
<td>Venous ammonia</td>
<td>0.19</td>
<td>0.062</td>
</tr>
</tbody>
</table>

CHAPTER XII
DISCUSSION

Our results provide additional evidences of increased oxidative stress in overt HE and MHE, as expressed by altered serum glutathione peroxidase antioxidant activity and increased levels of lipid peroxidation. Moreover, we demonstrated a significant correlation between the levels of the aforementioned oxidative stress markers and the results of Child and MELD specific scales, as well as with the venous blood ammonia level.

We demonstrated decreased activity of GPX and increased serum levels of MDA, as a lipid peroxidation marker, but also increased specific activity of SOD. This could be explained by the fact that SOD is the first line of defense against oxidative stress.
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stress development. In this way, this increase could represent a compensatory process as a result of elevated superoxides levels, which were previously demonstrated at the mitochondria level in the ammonia-exposed rats (13).

SOD and GPX are critical antioxidant enzymes that act cooperatively in the metabolic pathway of free radicals, and altered activity of one of the enzymes without compensatory changes in the other enzymes may result in increased oxidative stress. Superoxide radical, spontaneously or with the catalyses of SOD, is converted to hydrogen peroxide, which is reduced to water and oxygen molecules by the catalyses of GPX (5).

The results suggest that SOD activity is increased in response to increased reactive oxygen species production. On the other hand, lack of an accompanying similar increase in GPX activity might be one of the contributors of the increased lipid peroxidation, thus increasing MDA levels. This could be extremely important, considering that currently the exact mechanism which results in the generation of increased free radicals in HE is not fully understood (21).

Furthermore, our results confirmed that oxidative stress is implicated in the pathogenesis of cirrhosis (22-23).

A significant increase in MDA concentration was seen in overt HE group, as compared to MHE group or non-HE group, suggesting that MDA could be a physiopathological marker for HE.

All this aspects demonstrating the importance of oxidative stress in HE led to the hypothesis of using anti-oxidants as potential treatment. It was very recently demonstrated that N-acetyl cysteine could have protective effects in a rat bile duct ligation-induced model of HE, as expressed through decreased lipid peroxidation and an increase in the activity of antioxidant enzymes, as well as a significant improvement in the activity of liver marker enzymes, restored structural morphology of
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Liver and a significant amelioration of the spatial memory/motor coordination and cortex or cerebellum structural deficits (24).

In the present study, using the battery of psychometric tests PHES, which is accepted as a standard diagnostic test for MHE in several countries (25,26), we showed that it represents an efficient tool in the evaluation of cirrhotic patients with mild cognitive deficits.

We found that glutamine load increases the performance of psychometric tests (PHES) for MHE diagnosis in patients with liver cirrhosis. Previous studies have reported that glutamine-induced increased levels of ammonia were associated with variable modifications of psychometric testing (27-30). The differences in psychometric testing results throughout reports in the literature could be explained by variations in the type of test used, diagnosis criteria, and the type and dose of glutamine.

Glutamine was administrated at a dose of 20 g, similar to that in some studies (29), but higher than what was being used in others (27,31). However, this dose was well tolerated and did not precipitate overt HE in our cirrhotic patients. In line with previous reports (27,29-30), glutamine load did not modify the psychometric testing results and ammonia levels in the controls, demonstrating that it is not implicated in the cognitive status of the healthy subjects.

We decided to perform OGC using arterial blood ammonia as, according to previous studies (32-34), it proved more relevant than the venous one in the assessment of HE. Our study showed that arterial ammonia does not represent a specific biological marker for the diagnosis of MHE. Moreover, glutamine does not improve its diagnostic performance, as showed by the AUROC value (0.54 at baseline and 0.53 post-glutamine load).
Post-glutamine PHES score, unlike PHES alone, was significantly associated with the development of overt HE (p=0.004), showing that OGC increases its prognostic value. Considering the increase in both diagnostic performance and prognostic value of post-glutamine PHES, this test could be an useful and better screening tool for MHE than PHES alone. However, its feasibility is limited by the lack of convenience and time constraints.

Since PHES evaluation has not yet been standardized in our country, we have used a standardized PHES scoring based on Spanish norms which could influence our classification. However, the presumed differences between norms of other countries were limited by the use of a control group. Future studies should definitely move towards finding a larger applicable set of norms for PHES scoring in representative samples.

In accordance with the published data (19,35-38), we demonstrated efficacy of rifaximine and lactulose in the treatment of MHE, as well as in the maintenance of remission from overt HE, with no significant differences between the therapeutic regimens.

Our results could represent a starting point for evaluating in future studies the cost-effectiveness and budget impact of competing therapies in HE.

The prevalence of MHE(46.52%) is concordant with previous studies (39-43), but higher than in others that included patients with mild or moderate hepatic disease (25,29). Ditisheim et al (29) reported a prevalence of 44% for MHE in patients with moderate or severe hepatic failure. These differences suggest the implication of multiple risk factors in MHE, besides the severity of hepatic failure. Thus, alcoholic etiology of cirrhosis, large esophageal varices and previous
episodes of overt HE could be major risk factors for MHE in our study.

In line with previous data (40,44,45), we showed that MHE and severity of liver disease are independently related to overt HE.

CHAPTER XIII
CONCLUSIONS AND FUTURE PERSPECTIVES
RESULTING FROM DOCTORAL RESEARCH

1. The battery of psychometric tests PHES (NCT-A și B, DST, SDT,LTT) is an useful tool in evaluation of the patient with liver cirrhosis for detecting mild cognitive impairments.

2. MHE had a high prevalence (46.52% ) in patients with cirrhosis and mild or moderate liver disease.

3. Our results provide additional evidences of increased oxidative stress in overt HE and MHE, as expressed by altered serum GPX antioxidant activity and increased levels of MDA, as a marker of lipid peroxidation.

4. The aforementioned oxidative stress markers showed significant correlation with the severity of liver disease.
according to Child and MELD scales, as well as with the venous blood ammonia level.

5. MDA could be a physiopathological marker for HE.

6. The results open up new perspectives in exploring the role of the antioxidants in preventing and treating HE.

7. In cirrhotic patients, an oral glutamine load improves the psychometric diagnostic performance for MHE (53.7% at baseline and 79.63% after glutamine load).

8. Arterial ammonia does not represent a specific biological marker for the diagnosis of MHE. Moreover, glutamine does not improve its diagnostic performance, as showed by the AUROC value (0.54 at baseline and 0.53 post-glutamine load).

9. MELD score (OR = 1.5187, 95% CI: 1.0690 – 2.1574, p = 0.0197) and MHE (OR= 2.0402; 95% CI, 0.9457 – 4.4015, p=0.0288) were independent predictors for the development of overt HE.

10. Considering the increase in both diagnostic performance and prognostic value of post-glutamine PHES, this test could be an useful and better screening tool for MHE than PHES alone.
11. Our results confirmed the efficacy of rifaximine and lactulose in the treatment of MHE, as well as in the maintenance of remission from overt HE, with no significant differences between the therapeutic regimens.

12. We found a better tolerability of rifaximine.

13. The results could represent a starting point for evaluating in future studies the cost-effectiveness and budget impact of competing therapies in HE.

SELECTIVE REFERENCES


event-related potential for the diagnosis of minimal hepatic encephalopathy: evidence that psychometric hepatic encephalopathy score is enough. 13th ISHEN, Padua; 2008 [abstract].


LIST OF SCIENTIFIC PAPERS PUBLISHED IN THE AREA OF RESEARCH

Papers published in national journals (B+)

ISI papers published in extenso
Roxana Irimia, Carol Stanciu, Camelia Cojocariu, Cătălin Sfarti, Anca Trifan. Oral glutamine challenge improves the performance of psychometric tests for the diagnosis of minimal hepatic encephalopathy in patients with liver cirrhosis. _J Gastrointestin Liver Dis_ 2013;22(3).

Oral presentations, posters
Roxana Irimia, Anca Trifan. Minimal hepatic encephalopathy- is it necessary to diagnose and treat it? Oral presentation- Gastroenterology Symposion, Iasi, 8-10 december 2011.

Chapter book