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Analyzing Three Different Cognitive Spheres (Memory, Reasoning and Verbal Ability): An Online Psychometric Battery for the Assessment of Covert Hepatic Encephalopathy in Patients with Cirrhosis

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Ce mémoire intitulé

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Résumé

L'encéphalopathie hépatique (EH) est une complication neurocognitive débilitante de la cirrhose qui affecte la qualité de vie et augmente le risque de décès. L'EH est divisée en EH minimale, définie comme subclinique et HE manifeste, diagnostiquée avec des symptômes cliniques.

Cette étude vise à effectuer une évaluation des troubles cognitifs chez les patients atteints de cirrhose en évaluant trois domaines cognitifs (mémoire, raisonnement et capacité verbale) et en interprétant les valeurs prédictives de ces tests pour identifier les patients à haut risque de développer leur premier épisode d'EH manifeste dans l'année.

Cette étude longitudinale prospective a inclus des patients sans antécédent d'EH, recrutés à la clinique d'hépatologie du CHUM entre janvier et octobre 2021. Chaque patient a complété l'évaluation cognitive en ligne Cambridge Brain Sciences (CBS) au départ, composée de 12 tests neurocognitifs, qui prend 45 minutes. Les scores des patients ont été comparés aux normes CBS appariés pour l'âge, le sexe et les années d'éducation.

Les scores moyens des patients (n=34, 61,7% hommes, âge moyen 60,7±8,5 ans) étaient inférieurs à la moyenne des normes dans tous les domaines cognitifs étudiés ($p < 0,05$), ainsi que des scores inférieurs dans 11 des 12 tâches cognitives réalisées. Vingt-deux patients (65%) ont échoué à au moins un test. Jusqu'en janvier 2022, 3 patients ont développé une EH manifeste et 1 patient a terminé l'étude sans développer d'épisodes d'EH. Sur les questionnaires de suivi, tous les 3 ont signalé des troubles du sommeil, de l'attention et de la mémoire, avant l'épisode. De plus, ils avaient des scores inférieurs dans 8 des 12 tests cognitifs au départ.

L'évaluation cognitive CBS en ligne est facile à utiliser et réalisable. Il semble être assez sensible car la plupart des patients ont obtenu de mauvais résultats par rapport aux normes. La valeur du CBS réside dans sa capacité à prédire l'EH manifeste chez une population de patients atteints de cirrhose, ce qui permettrait d'identifier les patients à risque nécessitant un traitement et de prévenir de futurs épisodes d'EH manifeste.

Mots-clés : encéphalopathie hépatique, tests neurocognitifs, cirrhose, plateforme en ligne, Cambridge Brain Sciences.

Abstract

Hepatic encephalopathy (HE) is a debilitating neurocognitive complication of cirrhosis that impacts quality of life and increases the risk of death. HE is divided into covert, defined as subclinical HE and overt HE, diagnosed with clinical symptoms.

This study aims to perform a detailed assessment of cognitive impairment in patients with cirrhosis by evaluating three different cognitive domains (memory, reasoning and verbal ability) and interpreting the predictive values of these tests in identifying patients who are at high risk of developing their first episode of overt HE within one year.

This prospective longitudinal study included patients with cirrhosis without a history of HE, recruited from the CHUM hepatology clinic between January to October 2021. Each patient completed the Cambridge Brain Sciences (CBS) online cognitive assessment at baseline, composed of 12 neurocognitive tests, which required 45 minutes. Patient scores were compared to CBS controls matched for age, sex and years of education.

The patients (n=34, 61.7% male, average age 60.7±8.5 years) mean scores were lower than the average of the norms in all the cognitive domains studied ($p < 0.05$), as well as lower scores in 11 of 12 cognitive tasks performed. Twenty-two patients (65%) failed at least one test. Up until January 2022, 3 patients developed overt HE and 1 patient completed the study without developing any episodes of overt HE. On follow-up questionnaires, all 3 reported impairments in sleep, attention, and memory, leading up to the HE episode. In addition, they had lower scores in 8 of 12 cognitive tests at baseline.

The CBS online cognitive assessment is easy to use and feasible. It appears to be quite sensitive as most patients did poorly compared to controls. The value in the CBS lies within its ability to predict overt HE which would allow to identify patients at risk who require treatment and prevent future episodes of overt HE.

Keywords: hepatic encephalopathy, neurocognitive tests, cirrhosis, online platform, Cambridge Brain Sciences.

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List of abbreviations

AASLD- EASL: American Association for the Study of Liver Diseases- European Association for the Study of the Liver

ACLF: acute-on-chronic liver failure

AIH: Autoimmune hepatitis

ALD: alcoholic liver disease

ALT: alanine aminotransferase

ANA: antinuclear antibodies

APTT: activated partial thromboplastin time

ASMA: anti-smooth muscle antibodies

AST: aspartate aminotransferase

BCAA: Branched-Chain Amino Acids

CBS: Cambridge Brain Sciences

CGS: Coma Glasgow Scale

CHE: covert hepatic encephalopathy

CLD: chronic liver disease

CT: computed tomography

EASL: European Association for the Study of the Liver

ECM: extracellular matrix

EEG: electroencephalogram

HCC: hepatocellular carcinoma

HE: hepatic encephalopathy

HSC: hepatic stellate cells

ICU: intensive care unit

INR: international normalized ratio

ISHEN: International Society for Hepatic Encephalopathy and Nitrogen Metabolism

LKM-1: liver-kidney microsomal antibody type 1

LOLA: L-Ornithine-L-Aspartate

MELD-Na: MELD sodium

MELD: model for end-stage liver disease

MHE: minimal hepatic encephalopathy

MRI: magnetic resonance imaging

NAFLD: non-alcoholic fatty liver disease

NASH: non-alcoholic steatohepatitis

OHE: overt hepatic encephalopathy

OLT: orthotopic liver transplantation

PBC: primary biliary cirrhosis

PCR: polymerase chain reaction

PSC: primary sclerosing cholangitis

PT: prothrombin time

TE: transient elastography

TIBC: total iron-binding capacity

TIPS: trans jugular intrahepatic portosystemic shunts

SD: standard deviation

Science goes beyond our wish to find a specific result.
It's being open to finding the truth and learning how to use the facts to create solutions for the progression of multiple fields.

For those who dedicate their time to the advance of knowledge and apply it to the development of our society over time, offering one of the most valuable works.

Acknowledgments

The conclusion of this thesis is a consequence of a series of challenges in sequence.

When we start a journey toward an objective, we cannot always determine the course of the multiple events that will be involved in its achievement.

My gratitude extends to everyone who has helped me move forward with each step until the present moment.

I would like to thank my family and friends for the constant support and for understanding my wish to pursue my graduate studies and develop a career in another country.

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I would like to especially thank our research assistant, Mélanie Tremblay, for receiving me at the lab, always providing multiple instructions and valuable help during the project planning, execution and conclusion. She is one of the most capable and problem-solving persons I've met. Equally, I would like to thank Yvette Mukaneza, our research coordinator, for her excellence in keeping the quality of the project, for her useful advice and comments during all the phases of the project, and for her encouragement during difficult moments. All your help was very much appreciated. My special thanks are extended to all patients who participated in the project, wanting to contribute to the advancement of science despite their difficulties. I would like to thank my colleagues at the lab for sharing valuable information when needed, especially Amal Trigui, a valuable friend whom I had the joy of progressing side by side with.

Finally, I am grateful for my perseverance in facing challenges and for my constant belief that is possible to achieve our goals in life, if we have the patience and energy to develop ourselves according to the requirements. In the end, life is not just about what we achieve, but about the pathways we choose and the experiences we have during the trajectory.

Preface

Before starting with the introduction, it is important to mention that the present study was chosen and adapted due to the COVID-19 restrictions.

The original project was a multicentric longitudinal study, that intended to enroll around 25 cirrhotic patients per center, perform a cognitive assessment using the Stroop EncephalApp and collect blood samples at baseline at the hospital, including markers related to hepatic function, systemic oxidative stress and inflammation, as well as novel biomarkers. The patients would be followed for 1 year with trimestral phone calls, or until an episode of overt hepatic encephalopathy occurs. Results from the EncephalApp Stroop test, together with systemic biomarkers would be used to build a model of risk prediction of OHE.

In the impossibility of reaching the patients directly at the hospital because of the pandemic restrictions, we had to modify our study protocol and search for a new feasible option.

To replace the Stroop EncephalApp test, we opted for an online cognitive assessment, where the patients could perform the tests at home on their computers or tablets. To replace the blood samples at baseline, we used the most recent blood tests conducted and analyzed. We also decided the current project would be a single-center, longitudinal study, with one year of follow-up.

Due to the required changes, the beginning of the recruitment was delayed for at least 9 months, as all logistics involved in the design of the new project was modified, a new protocol of the study was amended, and we faced a new challenge in recruiting patients from home.

This study is still in progress and all 34 patients will finish their 1-year follow-up in October 2022. Unfortunately, due to time restrictions, this thesis will present the results obtained until January 31st, 2022.

Chapter 1 -Introduction

Cirrhosis, the end-stage of chronic liver disease (CLD), is one of the leading causes of mortality and morbidity across the world and adversely affects neurocognitive function. Hepatic encephalopathy, a neuropsychiatric and reversible complication associated to cirrhosis, can affect up to 80% of patients (Bajaj 2010).

These cognitive and altered mental status changes can range from subclinical and frequently undiagnosed impairments, collectively defined as covert hepatic encephalopathy (CHE) to overt hepatic encephalopathy (OHE), presenting with clinically evident symptoms including confusion, disorientation, asterixis to coma (Dharel and Bajaj 2015). The majority of OHE episodes require hospitalization and has a substantial impact on the risk of mortality. OHE also exerts an important impact on the quality of life of patients and caregivers and has been shown to lead to irreversible neurological damage in cases (Weiss and Thabut 2019). Understanding the significant impact of OHE on health outcomes, identifying patients with cirrhosis who are at risk of developing OHE remains an unmet care gap.

This study aims to investigate the cognitive performance of patients with cirrhosis by evaluating four different domains of cognition and understanding how cognitive alterations can impact daily activities, as well as predict the risk of the first episode of OHE.

The assessment comprises a full battery of neurocognitive tests, provided by Cambridge Brain Sciences (CBS), an online platform developed for research and healthcare providers. CBS involves scientifically rigorous and validated tasks and batteries which are very sensitive to alterations in multiple areas of cognition.

1.1 Chronic liver disease

1.1.1. Definition

CLD is a condition caused by different pathogenic factors. Independent of the etiology, continuous destruction of liver parenchyma ensues, progressing to fibrosis (represented by the excessive accumulation of extracellular matrix) and cirrhosis (an irreversible state resulting

from advanced fibrosis and presence of regenerative nodules), which significantly impacts liver function (Sharma and Nagalli 2022).

The progression of liver impairment is evaluated via multiple metabolic pathways linked to liver functioning, such as the decrease in circulating coagulation factors, albumin, increases in bilirubin and liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT).

The alterations from the normal values of these factors in the bloodstream, along with other metabolic alterations linked to the progressive pathological context of cirrhosis, can contribute to multiple liver-related complications found in clinical practice (Garcia-Tsao et al. 2010).

Cirrhosis of any etiology is also known to be the main risk factor for hepatocellular carcinoma (HCC) (Gomaa et al. 2008).

1.1.2. Etiology

The number of different etiologies of CLD is wide and ranges from toxins and medications to long-term alcohol abuse, infections, autoimmune diseases, genetic and metabolic disorders (figure 1). The most frequent cause of CLD is alcoholic liver disease (ALD), including alcoholic fatty liver disease and alcoholic hepatitis (being reversible with the abstinence of alcohol) (Sharma and Nagalli 2022). In association with metabolic syndrome, which includes the presence of obesity, hyperlipemia and diabetes mellitus, non-alcoholic fatty liver disease (NAFLD) may progress to non-alcoholic steatohepatitis (NASH) and consequent liver fibrosis and cirrhosis. Chronic hepatitis caused by viruses B, C, and D infections is also a common cause of CLD.

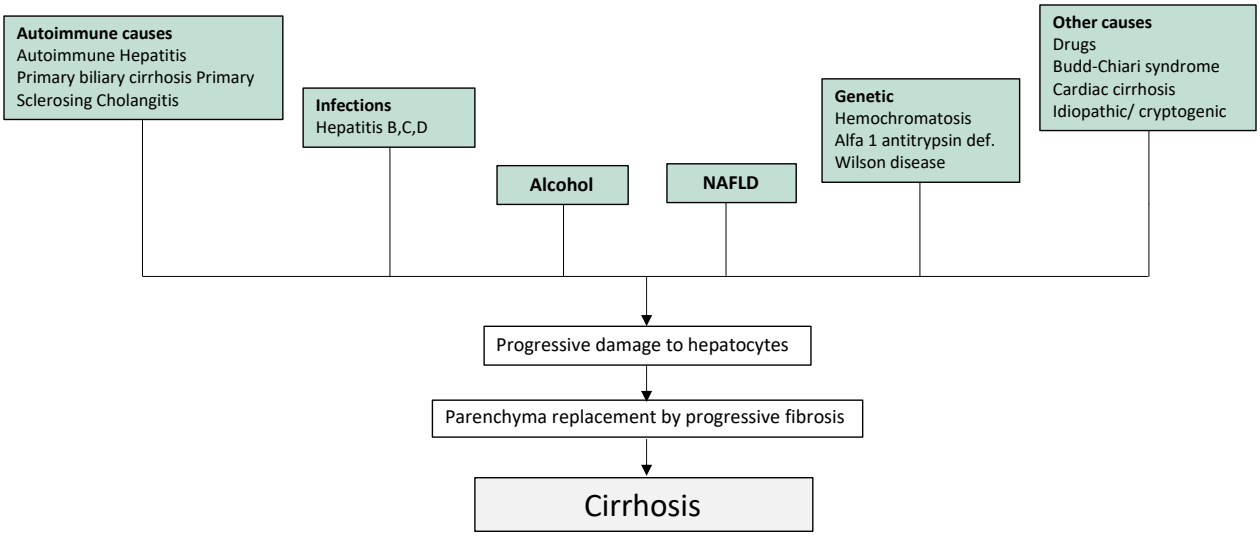


Figure 1. Etiologies of chronic liver disease. Multiple etiologies are responsible for progressive damage to the liver cells and consequent progression to fibrosis deposition and cirrhosis. NAFLD: Non-Alcoholic Fatty Liver Disease.

Among the genetic causes of CLD, there are: Hemochromatosis, characterized by increased iron levels in the body, Wilson disease, related to copper accumulation, and Alpha-1 antitrypsin deficiency, the most common genetic cause of CLD among children (Sharma and Nagalli, 2022).

The autoimmune causes of CLD include autoimmune hepatitis (AIH), where the liver parenchyma is destroyed by autoantibodies, primary biliary cirrhosis (PBC), defined by the destruction of the intrahepatic biliary ducts, and primary sclerosing cholangitis (PSC), characterized by a decrease in the size of intra and extrahepatic biliary ducts due to inflammation and fibrosis (Sharma and Nagalli, 2022).

Drug-induced liver injury can be classified into three patterns of injury: hepatocellular, cholestatic, and mixed hepatocellular-cholestatic. Drugs that can cause liver injury include amiodarone, used as antiarrhythmic; phenytoin, an anti-epileptic; methotrexate, an antimetabolite used as a chemotherapic and immunosuppressive drug; isoniazid, an antibiotic used to treat tuberculosis; nitrofurantoin, an antibiotic commonly used for urinary tract infections, and methyldopa, an antihypertensive drug. Each drug has a latency time to cause hepatotoxicity, which can range from days to months (Hayashi and Fontana 2014).

Acetaminophen, an analgesic/ antipyretic, is the main cause of drug-induced acute liver failure (Lee 2013).

There are other less common causes of CLD, such as Budd-Chiari syndrome, a rare disorder caused by obstruction of the hepatic veins by a blood clot, and cardiac cirrhosis, characterized by chronic hepatic congestion, secondary to cardiac dysfunction (Wells and Venkatesh 2018).

If an etiology cannot be determined by a lack of clinical, histological, laboratory or imaging parameters, cirrhosis is defined as idiopathic or cryptogenic.

1.1.3. Epidemiology

The CLD prevalence has increased over the years, contributing as a common cause of death, mainly in developing countries (Moon, Singal, and Tapper 2020) (figure 2).

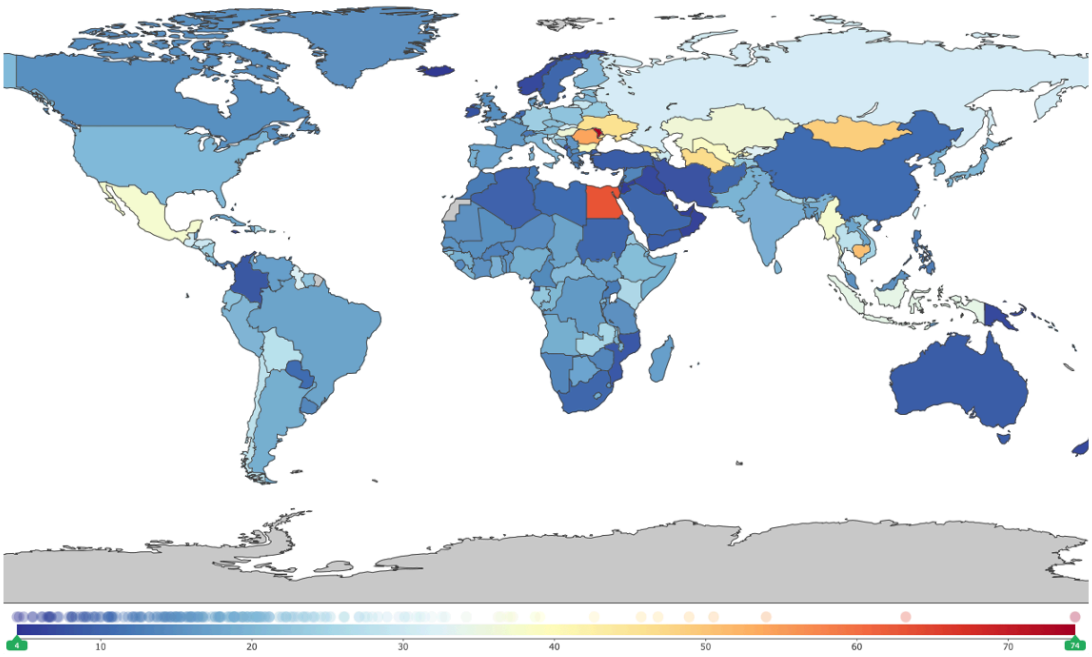


Figure 2. Global impact of cirrhosis mortality. Deaths per 100,000, both sexes, all ages due to cirrhosis and other chronic liver diseases in 2019. Legend: Dark to light blue (0-40). Yellow (40-50), Yellow- Orange (50-60), Orange- Red (60-70), Red (>70). Image adapted from (Sepanlou et al. 2020). Global Burden of Disease Study. https://vizhub.healthdata.org/gbd-compare/. Assessed 01/02/2022.

Statistics show that the number of cases of cirrhosis around the world can expand up to 1.5 billion and cause close to 2 million deaths each year. In addition, CLD can impact the health care systems and cause an increasing burden of disability for patients, impacting their labour capacities and, therefore, affecting families and society.

In 2016, CLD was the 11th cause of death and the 15th cause of morbidity worldwide, contributing to 2.2% of deaths and 1.5% of disability-adjusted life-years worldwide (Cheemerla and Balakrishnan 2021). In 2017, CLD accounted for approximately 1.32 million deaths, affecting predominantly men, who represented around two-thirds of the case numbers (Sepanlou et al. 2020).

In the United States, according to the National Vital Statistics Report 2017 from the Center for Disease Control and Prevention, approximately 4.5 million people had CLD and cirrhosis, accounting for 1.8% of the adult population. In the same year, these health issues were responsible for 41,473 deaths (12.8 deaths per 100,000 population) (Kochanek et al. 2019).

In Canada, according to the Canadian Liver Foundation (CLF) (2013), it was estimated that one in ten Canadians, or more than three million people, had some form of liver disease. Recently, CLF states that 1 in 4 Canadians may be affected, from newborns to adults.

The incidence of cirrhosis has increased importantly over the past 20 years in Canada. For example, the mortality rates show a drastic increase of 25% between the years 2000 and 2018, when cirrhosis and CLD were the 5th leading cause of mortality in the population aged 35-64 years. Global cirrhosis incidence is projected to increase in 2040 by a further 9% to 112.1/100,000 people, equally in both genders (Flemming et al. 2021).

The major number of CLD cases in the developed countries is represented by the following etiologies: alcoholic liver disease, chronic viral hepatitis (B and C), NAFLD, and hemochromatosis (Heidelbaugh and Bruderly 2006). Viral hepatitis B is the primary cause in China and other Asian countries (Asrani et al. 2019).

Traditionally, viral hepatitis has been one of the main causes of CLD and cirrhosis. However, improved prevention techniques like vaccination (in the case of hepatitis B) and treatment (in the case of hepatitis C) have led to changes in historical CLD trends. Nevertheless,

obesity and alcohol consumption, which are not uncommon and increasing worldwide, have become key liver disease risk factors (Cheemerla and Balakrishnan 2021).

1.1.4. Pathogenesis and consequences of chronic liver disease

1.1.4.1. Pathogenesis

CLD promotes a state of prolonged damage to the liver, causing histological distortion and progressive fibrosis, resulting in cirrhosis, a final stage, that can lead to a higher risk of hepatocellular carcinoma, primary cancer of the liver.

The pathogenesis of CLD involves multiple mechanisms, such as inflammation, hepatic stellate cell activation, angiogenesis, and fibrogenesis.

Hepatocytes, the main parenchymal cells of the liver, are the targets for many hepatotoxic agents, represented by the multiple etiologies of cirrhosis. In CLD, the continuous damage to these cells can promote apoptosis, the process of programmed cell death, or trigger a mechanism of regeneration, that can activate hepatic stellate cells (HSC) and stimulate fibrogenesis (Zhou, Zhang, and Qiao 2014).

HSC, pericytes located in the perisinusoidal space of the liver (space of Disse), can transform into an activated state following insults and injury, and which play a central role in the initiation and progression of fibrosis deposition in the live (Zhou, Zhang, and Qiao 2014). This process is the underlying cause leading to cirrhosis formation, resulting from the deposition of proteins in extracellular matrix (ECM) in response to chronic liver injury, independent of the etiology. HSC are stimulated by pathologic factors present in CLD, like chronic inflammation, cytokine production by damaged parenchymal cells, or disruption of the extracellular matrix.

When activated, the HSC get modified into a myofibroblast-like phenotype, upregulating the expression of certain inflammatory receptors and mediators, by releasing chemokines and other leukocyte chemoattractants. This initial process marked by pro-inflammatory changes also modifies gene and phenotypic expression of the liver cells, making them more responsive to these inflammatory cytokines. The prolongation of this process is responsible for the accumulation of ECM, explaining the pathology of progressive fibrogenesis in the liver (Tsuchida and Friedman 2017)(figure 3).

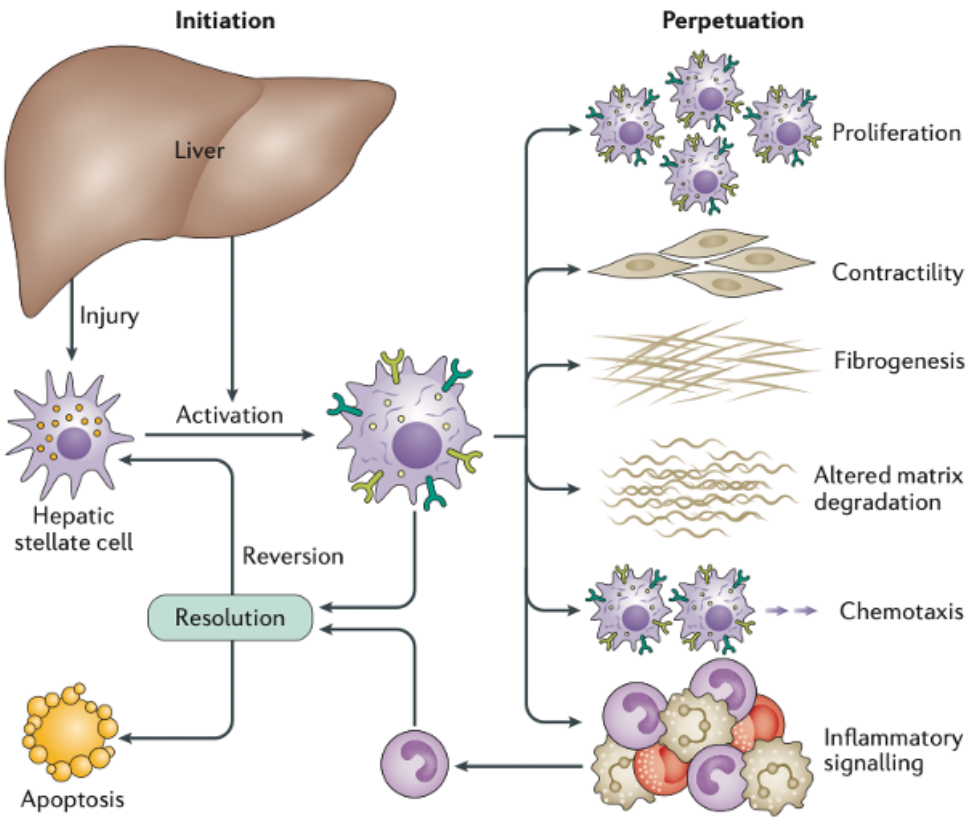


Figure 3. Hepatic Stellate Cells play a central role in the pathogenesis of liver cirrhosis. Liver injury by different etiologies causes activation of hepatic stellate cells (HSC), which can progress to a perpetuation phase, that goes through proliferation, contractility, fibrogenesis, altered matrix degradation, chemotaxis and inflammatory signalling, resulting in scar formation. The process of HCS clearance includes the reversion to the inactivated state or apoptosis. Image from (Tsuchida and Friedman 2017).

When cirrhosis is established, there is an unusual disruption of liver architecture, represented by the presence of regenerative nodules in the parenchyma, deposition of ECM and consequent reorganization of the vascular system in the liver, with neo-angiogenesis (formation of new blood vessels) (Sharma and Nagalli 2022). This damage remains reversible if it does not exceed the regenerative capacities of the liver.

Different etiologies of CLD can produce distinct patterns of liver fibrosis. Periportal and septal fibrosis are found in the presence of hepatotropic viruses, while centrilobular and perivenular distribution are associated with sinusoidal fibrosis in ALD and NAFLD (Poynard et al. 2003).

The process of fibrogenesis in consequence of continuous parenchymal damage is usually irreversible, except for the initial stage. The modification to irreversibility is still not completely understood and there is no time point clearly defined for the transition. However, in the absence of effective treatment for CLD, the common endpoint is usually permanent fibrosis, regeneration nodule formation, and development of liver cirrhosis.

1.1.4.2. Consequences of CLD

When the diagnosis of cirrhosis is established, the complications are explained by the progressive loss of hepatocyte function and increasing pressure in the portal vein (the main vessel that drains blood from the gastrointestinal system into the liver and then back to the systemic circulation). Further alterations related to the gut-liver axis and inflammation can also play an important role in pathophysiology.

Loss of liver functions

As the healthy cells are progressively replaced by scar tissue, the liver decreases its capacity to perform its functions. The liver failure resulting from this process causes impairments in multiple metabolic pathways, leading to:

a) increase in ammonia, a neurotoxin produced by bacteria in the gut, resulting from the protein digestion,

b) alteration in liver enzymes, such as ALT, AST, GGT and alkaline phosphatase (ALP), which participate in multiple metabolic functions,

c) altered bile excretion (a substance produced by the liver, that participates in fat digestion and excretion of substances,

e) decreased in the synthesis of coagulation factors, that stops bleeding,

f) decrease of production of albumin, an abundant protein in the bloodstream and tissues that maintain the osmotic pressure, bind and transport multiple substances in the body.

These multiple alterations can cause clinical manifestations, which will be explained in more detail in chapter 1.1.7.

Portal hypertension

When the disease is established, the liver becomes granular and stiff due to structural modifications associated with fibrosis, increasing resistance to blood flow. The result is a direct increase in the portal vein pressure and its collaterals due to an accumulation of blood. Following this pathological modification, the resulting portal hypertension can cause multiple complications, such as ascites and gastrointestinal varices. The high pressure in the portal vein might result in reversal flow through collateral veins, making the blood bypass the liver and go directly to the systemic circulation.

Normally, the blood from the gastrointestinal system is filtered by the liver, which metabolizes toxins such as ammonia. The formation of portosystemic shunts, abnormal bypass between portal vein blood and systemic circulation due to progressive portal hypertension, allows unfiltered blood to circulate the body carrying ammonia. Reaching the brain, this neurotoxin can damage brain tissue, contributing to the development of HE, along with other pathological pathways (chapter 1.2.2.2).

The role of gut flora and inflammation

Alterations in gut flora related to CLD facilitate bacterial translocation, the passage of bacteria or bacterial by-products through the gut mucosa to the systemic circulation, which can produce secondary systemic inflammation, contributing to decompensation, a clinical state marked by the presence of liver-related complications, such as ascites, variceal bleeding and HE (Acharya and Bajaj 2019).

There is also evidence that systemic inflammation is involved in the acute development of other complications, like ascites and kidney failure (Tandon and Garcia-Tsao 2008).

Besides, the presence of portosystemic shunting, which can occur as a consequence of portal hypertension and reversed blood flow, impairs the normal process of gut-derived bacteria and their subproducts to be eliminated from the portal system by the liver, which contains about 90% of the reticuloendothelial cells in the body (Tandon and Garcia-Tsao 2008).

CLD patients are also in an immune dysfunction state, known as cirrhosis-associated immune dysfunction syndrome (Bonnel, Bunchorntavakul, and Reddy 2011; Bunchorntavakul and Chavalitdhamrong 2012). The increased risk of infections is justified by synergism between a state of excessive activation of pro-inflammatory cytokines associated with an impaired immune response.

Most components of the immunologic system are significantly impaired in cirrhosis, resulting in a decrease in phagocytic activity and in serum albumin, complement and protein C activities, and an impaired opsonic activity both in serum and ascitic fluid (Shawcross et al. 2008; Wasmuth et al. 2005). Beyond these factors, cirrhosis-associated immune dysfunction may further complicate by additional factors such as malnourishment (Ledesma et al.,1992) and alcohol drinking (Gomez, Ruiz, and Schreiber 1994).

Bacterial infections in cirrhosis, associated with the dysregulated cytokine response state, transforms helpful responses against infections into excessive, damaging inflammation (Gustot et al. 2009), what can facilitate other mechanisms of disease progression and decompensation.

A summary of the progression of CLD, which is a result of the mechanisms previously explained, is shown in figure 4.

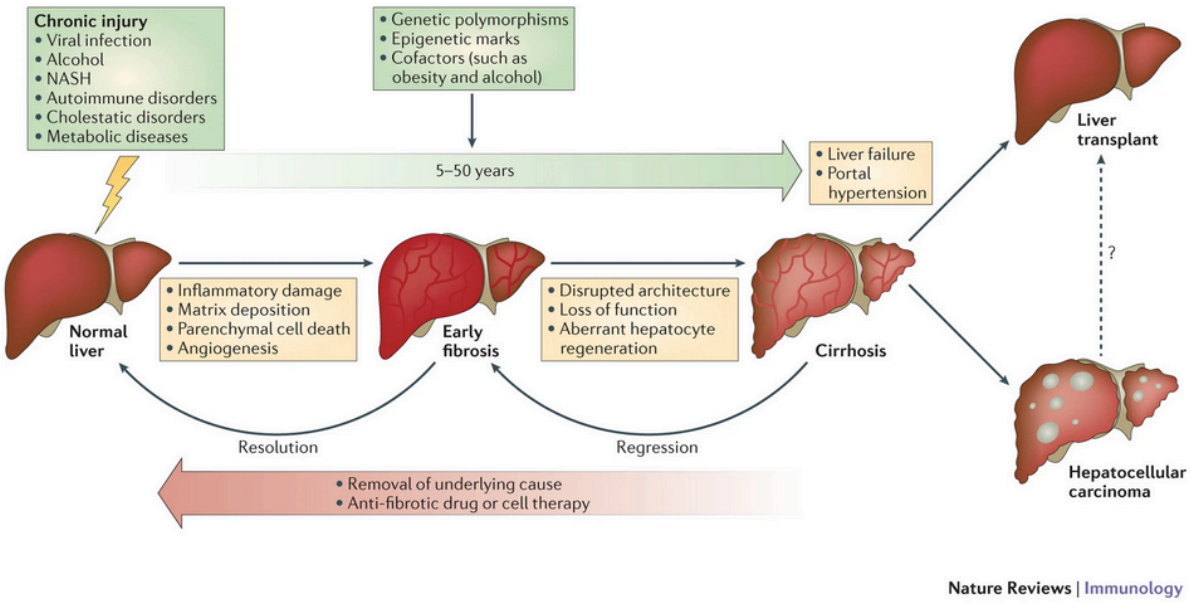


Figure 4. The natural progression of chronic liver disease. Independently of the etiology, chronic injury during a variable time, ranging from a few years to decades, can promote a progressive state of liver damage, cursing with parenchymal, vascular, and functional alterations that, ultimately, can lead to anatomic and physiological dysfunctions in the liver and other tissues. The final states include the evolution to cirrhosis and hepatocellular carcinoma. Image from (Pellicoro et al. 2014).

1.1.5. Diagnosis of liver disease

The liver biopsy is the gold standard procedure for the evaluation of liver fibrosis and cirrhosis. However, it remains an invasive method to assess histology and severity.

To reach the liver tissue and obtain the material for a biopsy, there are different current techniques available, like a percutaneous, transjugular or laparoscopic biopsy, and fine-needle aspiration guided by ultrasound or computed tomography (CT) (Bravo, Sheth, and Chopra 2001).

However, an initial step consists in identifying the primary cause of CLD. Considering the patient's symptoms, specific exams can be performed to identify a primary cause. For example, diagnostic of viral hepatitis B and C requires serology and PCR (quantitative and qualitative) with genotype, while elevated levels of AST in comparison to ALT with a history of

chronic alcohol intake are required for the diagnosis of alcoholic liver disease. Raised serum iron, ferritin, and decreased total iron-binding capacity (TIBC) are used for the diagnosis of hemochromatosis. NAFLD is found in the presence of ALT levels higher than AST and it is usually a diagnosis of exclusion.

Raised urine copper, decreased serum ceruloplasmin and genetic testing for ATP7B gene are required for Wilson disease; while raised alkaline phosphatase levels with an antimitochondrial antibody are required for primary biliary cirrhosis diagnostic.

Decreased levels of alpha 1 antitrypsin are found in alpha 1 antitrypsin deficiency; raised antinuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), and liver-kidney microsomal antibody type 1 (LKM-1) are diagnostic tests for autoimmune hepatitis.

1.1.5.1. Laboratory Findings

In CLD, there are higher levels of AST and ALT released in the bloodstream, which can be explained by the presence of inflammatory modifications and hepatocytes damage (Ellis et al. 1978).

As a consequence of progressive liver failure and consequent hepatocellular insufficiency, some alterations can be found. For example, we might find a decrease in albumin and an increase in ammonia levels, further alterations include increased bilirubin (unconjugated > conjugated) levels, which can cause jaundice, and reduced production of coagulation factors, raising prothrombin time (PT), international normalized ratio (INR) and activated partial thromboplastin time (APTT), clinically leads to an increased risk of bleeding.

The clinical complications will be explained in section 1.1.7.

Usually, laboratory alterations can reflect the severity of hepatocyte dysfunction and tend to be more pronounced as the hepatic insufficiency progresses.

1.1.5.2. Radiologic Investigations

Imaging diagnostic tools are widely used in CLD as non-invasive techniques. They have easy applicability in clinical practice and contribute to the diagnostic, as well as indicate the need for a subsequent liver biopsy. Among these exams, we can mention:

a) Ultrasound: one of the most used and accessible imaging techniques for detection of liver size and echogenicity nodularity, being able to identify the main features of liver cirrhosis.

b) Computed tomography (CT) scan: a more precise imaging study, that can show lesions in the liver in more detail or even the presence of obstruction of the biliary tract.

c) Transient elastography (TE) or FibroScan® (FS): a non-invasive technique used to assess hepatic fibrosis by the measure of liver stiffness.

Radiological studies can also detect possible complications (e.g.: ascites, portal hypertension and splenomegaly), particularly in the advanced stages of cirrhosis.

The clinical signs of portal hypertension and liver insufficiency (e.g., gastrointestinal varices, jaundice, HE, ascites) are also part of the diagnosis of cirrhosis.

1.1.6. Progression: compensated, decompensated and end-state chronic liver disease

As previously explained, the progression of liver disease encompasses a continuous process of inflammation, destruction, and regeneration of liver parenchyma, which leads to fibrosis and cirrhosis. Different disease states, such as compensated and decompensated cirrhosis, are related to the progression of these mechanisms.

The compensated state is the asymptomatic phase, where the disease is not yet clinically manifested. After the establishment of cirrhosis, a variably long asymptomatic phase usually precedes the later states, and this time depends on the identification and treatability of the primary cause of liver damage.

The end-stage of the liver disease progresses over months, years, or even decades after the establishment of cirrhosis. At this stage, the clinical condition can deteriorate despite the treatments. The decompensated state is marked by the current or previous presence of cirrhosis-related complications, including ascites, gastrointestinal bleeding, jaundice or HE

(D'Amico et al. 2018). Furthermore, the decompensated state can be presented by a further worsening of liver dysfunction (D'Amico et al. 2018).

The ultimate consequence of cirrhosis is the development of hepatocellular carcinoma, in which in some cases, the only option to prolong survival might be orthotopic liver transplantation (OLT).

A scheme of these states representing the course of CLD is demonstrated in figure 5.

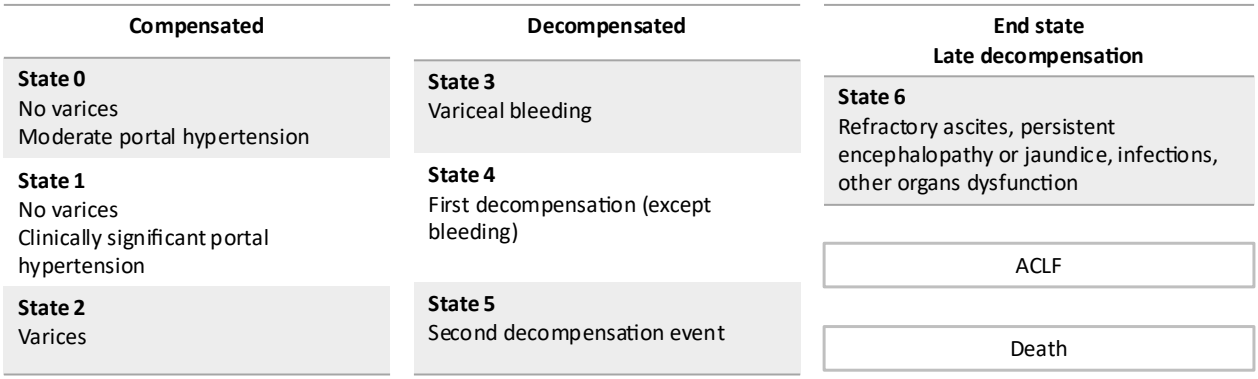


Figure 5. The course of chronic liver disease. The multiple stages according to the severity of liver dysfunction. The progression is not always linear. Clinically significant portal hypertension is defined by a hepatic venous pressure gradient ≥10 mmHg. ACLF: Acute-on-chronic liver failure. Adapted from D'Amico et al., 2018.

The progression of CLD is determined by multiple factors, that can exert a synergistic effect towards advanced levels of severity. Progressive portal hypertension, increased oxidative stress, bacterial translocation, inflammation (and neuroinflammation), as well as hyperdynamic circulation are factors that usually coexist in the context of cirrhosis and are well-known mechanisms of decompensation (Bernardi et al. 2015).

Histological stages of cirrhosis, fibrosis level and nodules size are important determinants of portal hypertension levels and, therefore, predictors of clinical severity of the disease (Sethasine et al. 2012; Rastogi et al. 2013).

1.1.7. Clinical complications of cirrhosis

The clinical manifestations of cirrhosis are extensive, and they develop according to the progression and severity of the underlying liver disease, which can lead to multiorgan dysfunction and to the multiple mechanisms of decompensation (D'Amico et al. 2018).

Even if the hepatic histology shows common patterns of fibrosis after the establishment of cirrhosis, the patients' symptomatology and complications may vary significantly according to the etiology of the disease, as well as the risk and time to develop them.

Among the general symptoms found in cirrhosis, the patients can experience loss of appetite, fatigue, and muscle wasting. Other manifestations include telangiectasias, palmar erythema, white nails, spider angiomata, finger clubbing and others.

The main clinically significant complications are caused by portal hypertension and hepatocellular insufficiency. CLD, mainly during the decompensated phase, can present with the main following complications and consequences (figure 6):

a) Esophageal varices/bleeding: The bleeding event can externalize through melena or hematemesis (presence of the blood in feces or vomit, respectively), as a consequence of portal hypertension. This is the most life-threatening complication of cirrhosis, and the risk increases according to the varices calibre and presence of red signs (endoscopic signs suggesting a high risk of variceal bleeding) or Child-Pugh B-C class, which represents moderate to advanced levels of liver dysfunction (Garcia-Tsao and Bosch 2015).

b) Ascites: It is an accumulation of fluid in the peritoneal cavity because of increased hydrostatic pressure resulting from raised portal pressure, associated with decreased albumin (reduced oncotic pressure), and splanchnic vasodilation (due to the release of nitric oxide). Ascites is a hallmark of decompensation and is associated with five-year mortality of around 50% (Zipprich et al. 2012).

c) Hepatic Encephalopathy: A neuropsychiatric syndrome associated with CLD, that will be discussed in section 1.2.2.

d) Jaundice: is a yellowish coloration of the eyes, skin, and mucous membrane because of overproduction or under clearance of bilirubin, which deposits in various tissues of the body as a consequence of liver disease, where bilirubin is not conjugated properly.

Figure 6. Cirrhosis-related complications. Alterations are found in multiple organs or systems and are associated with the underlying liver disease**.** Figure created in BioRender.com

e) Spontaneous Bacterial Peritonitis (SBP): Condition when bacteria from the gastrointestinal tract (E. coli, Klebsiella, Streptococcus pneumoniae) seep and cross the intestinal wall and infect the ascitic fluid. The infection spreads through the fluid to the peritoneal membrane, causing inflammation. SBP presents with fever, generalized abdominal pain, tenderness, and absent bowel sounds.

f) Hyperestrogenism: In CLD the catabolism of estrogen becomes impaired, resulting in excess estrogen in the body. This alteration manifests as palmar erythema, spider angiomas (dilated cutaneous arterioles with a central red spot and red extensions that radiate outward like a spider's web), gynecomastia (enlarged tender subareolar tissue), and testicular atrophy.

g) Hepatorenal Syndrome (HRS): is represented by the development of renal failure in the context of advanced liver disease, due to severe renal vasoconstriction. The result is a decrease in the glomerular filtration and progressive oliguria (decreased production of urine), causing a poor prognosis.

h) Hepatopulmonary syndrome (HPS): is characterized by abnormal arterial oxygenation caused by intrapulmonary microvascular vasodilation in the context of portal hypertension or congenital portosystemic shunts.

i) Splenomegaly: enlargement of the spleen because of portal hypertension, holding higher amounts of blood cells, reducing the numbers of these cells in the circulation.

j) Coagulopathy: Represented by the deficiency in the production of clotting factors, represented by prolonged PT/INR (intrinsic coagulation pathway, activated by exposed endothelial collagen) and APTT (extrinsic coagulation pathway, activated by tissue factor released by endothelial cells after external damage), what contributes to bruising and bleeding (Chaudhry, Usama, and Babiker 2022).

k) Infections: common in advanced cirrhosis, due to bacterial translocation, driven by the progression of portal hypertension and liver dysfunction (Bajaj, O'Leary, et al. 2012). Cirrhotic patients are in a state of immune dysfunction, associated with an excessive inflammatory response, predisposing the patient to infections, as explained in section 1.1.4. Mortality may reach 38%, while discharged patients have a 30-day readmission rate of 35% and six-month mortality of 23% (Piano et al. 2017).

l) Sarcopenia: a deterioration in muscle quantity and quality, that decreases muscle functional capacity, is a very common finding and an independent prognostic factor for survival (Montano-Loza et al. 2012).

m) Cancer: cirrhosis is also the main risk factor for liver cancer (hepatocellular carcinoma), which represents around 90% of all primary liver cancers, and is one of the leading causes of cancer-related death in the world (Yang and Heimbach 2020).

1.1.8. Classification and prognostic models

Prognostic models are commonly used in the care of patients with cirrhosis to assess severity and mortality risk, such as the Child-Pugh (also known as Child-Turcotte-Pugh) score (table 1), the Model for End-stage Liver Disease (MELD) score, and the MELD-Sodium (MELD-Na) score.

Child- Pugh Score			
Parameter	**1 point**	**2 points**	**3 points**
Albumin (g/dL) (g/L)*	>3,5 >35	2,8- 3,5 28-35	<2,8 <28
Total bilirubin (mg/dL) (µmol/L) *	<2 <34.2	2- 3 34.2-51.3	>3 >51.3
Prothrombin time (s) INR	1- 3 <1,7	4- 6 1,7- 2,3	>6 >2,3
Ascites	none	slight	moderate to severe
Encephalopathy	absent	grade 1-2	grade 3-4

Class	Points
Child- Pugh A	5- 6
Child- Pugh B	7- 9
Child- Pugh C	10- 15

Table 1. Child-Pugh classification and score of cirrhosis. The scoring system increase according to the severity of the liver disease and is calculated based on the sum of the individual scores of the 5 items, as above. INR: International normalized ratio. * Units of measure standardized at CHUM were added to the table: g/L (albumin) and µmol/L (total bilirubin).

The Child-Pugh score was initially created to predict cirrhosis-related mortality (Tsoris and Marlar 2022). It is calculated from the sum of 3 blood markers and 2 clinical manifestations: total bilirubin, albumin and prothrombin time, ascites and HE. Each item receives points according to pre-determined values, presence or absence or stage grading, ranging from 1 to 3.

The scores 1, 2 and 3 indicate low, moderate or high damage, respectively. The final classification reflects the sum of the cumulative points, classifying cirrhosis into three categories:

A (5 to 6 points): good hepatic function

B (7 to 9 points): moderately impaired hepatic function

C (10 to 15 points): advanced hepatic dysfunction

The MELD score was initially used to predict mortality in patients post TIPS (transjugular intrahepatic portosystemic shunts) placement (Malinchoc et al. 2000). As an excellent predictor of 3-month mortality, it is a useful score to prioritize patients for receiving OLT (Wiesner et al. 2003).

The MELD score is based on 3 biochemical variables that are readily available, reproducible, and objective: serum bilirubin, serum creatinine, and the international normalized ratio of prothrombin time. The mortality risk increases according to higher scores (table 2).

MELD-Na Score	90-day mortality
<17	<2%
17-20	3-4%
21-22	7-10%
23-26	14-15%
27-31	27-32%
≥32	65-66%

Table 2. The MELD-Na score for end-stage liver disease. Higher MELD-Na scores correspond to a progressive risk of mortality in 3 months. Adapted from Kim et al., 2008.

The MELD-Na score, which includes sodium in addition to the other elements mentioned above, was developed to consider the additional risks associated with hyponatremia. The MELD-Na score has been shown to be better suited to predict mortality than the MELD score alone (Biggins et al. 2006).

1.1.9. Treatments

The treatment of CLD requires a multidisciplinary approach and consists, primarily, in the treatment of the underlying etiology, associated with the management of complications related to CLD and portal hypertension.

For the correction of the primary cause, there are multiple measures and treatments available, for example: chronic viral hepatitis can be treated with antiviral drugs, while alcohol abstinence is indicated for ALD. For NASH, weight control using healthy diets and physical activity can slow the progression of liver damage.

Concerning the treatment of liver-related complications, procedures like endoscopic variceal banding can be used to manage variceal bleeding, as well as ascites punctions to treat voluminous ascites and TIPS for portal hypertension treatment. Measures like the use of vaccines can be used to prevent certain viral infections.

In the case of progression to hepatocellular carcinoma, the treatment includes surgery, thermoablation and chemoembolization.

And finally, OLT is the only curative treatment for end-stage liver disease, in the case of decompensated cirrhosis or severe liver failure, in selected patients.

1.2. Cognitive function in chronic liver disease

1.2.1. Cognitive alterations

Cognition can be divided into multiple different brain functions, depending on the anatomic areas or circuits involved. It is represented by a diversity of mental processes, associated with the capacity of thinking, understanding, acquiring information, as well as storing, retrieving and manipulating it according to the circumstances.

Cognitive processes include attention, working memory, evaluation, judgement, problem-solving, decision making, comprehension, language production, reasoning and others (Sadkhan 2018). Cognitive dysfunction refers to the presence of impairments in these processes, manifesting as a range of mental alterations not found in a healthy state or related to the

expected decline of normal aging. A mild decline in cognitive abilities might not be noticeable and, in consequence, undiagnosed.

Cognitive impairments are present in approximately 80% of patients with cirrhosis (Das et al. 2001; Ortiz, Jacas, and Cordoba 2005), and are associated with multiple negative outcomes.

Studies have shown that alterations in psychometric tests reflect the severity of liver disease and are associated with increased risk of death. The presence of cirrhosis, independent of age and educational levels, was confirmed to negatively influence mental functions in comparison to controls (Amodio et al. 1999). Cognitive dysfunction and previous OHE episodes were also associated with worse employment, financial difficulties and caregiver burden (Bajaj et al. 2011).

The cognitive domains usually impaired include alertness and orientation, reaction times and psychomotor speed, vigilance and sustained attention, and executive functions, which involve working memory, judgement, planning, response inhibition and problem-solving capacities (Bajaj et al. 2014). The profile of cognitive alterations found in cirrhosis may include impairments in some or multiple of these domains, what can make the diagnosis challenging in subclinical phases of HE, especially because the traditional psychometric tests were developed to assess these domains, but most of them don't access all the range of cognitive alterations simultaneously.

Due to its association with poor prognosis in cirrhosis, screening for cognitive alterations is recommended by North American and European liver disease societies (Vilstrup et al. 2014). However, studies show that approximately only 10% of health care providers screen patients for alterations in cognition due to a lack of time and consensus on the methodology as well as qualified staff required to apply and interpret the results of the tests (Shawcross et al. 2016).

Unfortunately, multiple neuropsychological and neurophysiological tests used in research do not transition to clinical practice because of costs of equipment, long test times as well as the difficulty to find a consensus on a cut-off to define an abnormal result relative to the healthy population (Morgan et al. 2016; Patidar et al. 2014). So far, this remains a hindrance to testing patients for CHE.

It is important to mention that there is a range of focal deficits in patients with cirrhosis that may be unrelated to HE, which may be due to (1) previous alcohol abuse, resulting in neuropathy and cerebellar alterations (Groeneweg et al. 2000), (2) direct effects of cirrhosis on the nervous system, such as hepatic myelopathy and extrapyramidal signs (Jover et al. 2003), (3) deficits unrelated to liver disease, like previous deficits caused by strokes, and (4) diseases that affect the brain and liver simultaneously, such as Wilson's disease (Ferenci 2005). Furthermore, other studies confirm that different etiologies, like NAFLD, viral hepatitis and others, may affect the brain independently of liver failure itself (Balzano et al. 2018; Forton et al. 2001).

The clinical identification and psychometric profile of all these pathologies with neurological manifestations is not always obvious, and often the diagnosis may not be clear, requiring the use of multiple tools to identify it, as specific psychometric tests, imaging studies, and laboratory exams.

A diagnosis of HE can only be made after ruling out other possible causes of brain dysfunction, and distinguishing HE from other acute or chronic causes of altered mental status can be challenging in CLD. The responsiveness of patients to first-line treatment for HE usually reinforces the diagnosis, when the lack of response associated with the presence of unusual neurologic findings may suggest a differential diagnosis and indicate the need for further neurologic studies (Weissenborn 2019).

Although other neurologic syndromes might be important in our overall understanding, HE remains the most prevalent neuropsychiatric complication of cirrhosis (Bajaj, Wade, and Sanyal 2009).

1.2.2. Hepatic encephalopathy

1.2.2.1. Definition, prevalence, and impact

HE is a vast neurological syndrome resulting from acute or chronic hepatic dysfunction and/or portosystemic shunting, whose complexity is manifested in the form of a spectrum of unspecific neuropsychiatric alterations, ranging from subclinical impairments to coma and, in

some cases, death (Dharel and Bajaj 2015) standing as one of the most significant complications of cirrhosis (Bajaj 2008).

HE classification is based on different grades of progressive severity, creating a subdivision that includes two main categories with different risk stratification: CHE, also known as minimal HE, and OHE. The HE classification will be further explained in section 1.2.2.4.

CHE represents the initial and subclinical stages and can be found in 20 to 80% of the patients according to several studies, depending on the methodology used for diagnosis and the population evaluated. OHE compasses the higher grades of severity, with evident clinical symptoms. The incidence of OHE is around 30 to 45% in cirrhotic patients, (Bajaj 2010), and around 25% of them develop OHE in the first 5 years after the diagnostic (Duarte-Rojo et al. 2019).

Usually, the syndrome occurs in advanced stages of liver disease and rarely is seen as one of the first decompensation events, but patients with a previous episode have a 42% risk of developing a subsequent episode in the next year (Sharma et al. 2009). OHE is associated with a 5-year survival rate of around 20% (D'Amico et al. 2014).

It is well known that CHE is associated with a meaningful impact on patients' quality of life and drive performance, in addition to increased risk of hospitalizations and death. Equally, OHE is importantly associated with (re)hospitalizations and mortality rates, and poor quality of life (Patidar and Bajaj 2015).

OHE places patients at a 2-fold risk of death during 1 year in comparison to patients without a previous episode, predisposing them to a median survival time of just a few months (Cordoba et al. 2014).

HE-related hospitalizations and consequent re-admissions also cause increased costs for healthcare systems (Tapper et al. 2016), and the high rates of mortality are unrelated to the severity of the CLD. This fact indicates that HE may have independent pathophysiological and prognostic implications (Cordoba et al. 2014) and results in the utilization of more healthcare resources, compared to other complications of CLD (Tapper et al. 2016). Furthermore, there is evidence that some patients can experience irreversible HE-related alterations, which can further contribute to increased unemployment rates and consequent financial burden and decrease in quality of life (Weiss and Thabut 2019).

Usually, HE is considered to be reversible, but factors such as neuroinflammation and neuronal cell death, that can be present in the syndrome, can lead to persistent neurocognitive dysfunction, especially in patients with multiple episodes. Studies have shown that the irreversibility due to these factors may persist after LT, being found in up to 47% of patients, which remains affecting their quality of life and adding to the economic burden on the healthcare systems (Garcia-Martinez et al. 2011) (Ochoa-Sanchez et al 2021).

1.2.2.2. Pathogenesis

As a complex and multifactorial clinical syndrome, the pathogenesis of HE is still not fully understood.

The central implication of ammonia in the pathological process of HE, as a neurotoxic substance capable of causing astrocyte swelling and brain dysfunction (Liere, Sandhu, and DeMorrow 2017), is a consensus in the literature, although its serum values are usually not used for the diagnosis of HE.

Ammonia is a by-product of the digestion of proteins in the gut as a part of the usual physiological process when the colonic bacteria and enzymes from the intestinal mucosa break down proteins from the diet, releasing ammonia to be absorbed into the portal circulation.

In healthy individuals, the liver converts ammonia into urea, a non-toxic substance that is removed from the circulation mainly by the kidneys. In the context of liver failure and/or portosystemic shunting, the ammonia released into the portal circulation does not get metabolized by the liver as usual and it accumulates at high levels in the systemic circulation (Prakash and Mullen 2010).

The consequence is a substantial increase in ammonia levels crossing the blood-brain barrier (BBB) and reaching the cerebral tissues. In the brain, the astrocytes will convert ammonia into glutamine, an amino acid used in the biosynthesis of other substances, like neurotransmitters. If the glutamine levels accumulate inside the astrocytes, the increase in osmolarity creates a favourable gradient for the passage of water into the cell, resulting in astrocyte swelling. The consequence of this process is cerebral edema, intracranial

hypertension, only found in acute liver failure and consequent neuronal dysfunction (Prakash and Mullen 2010).

It is also well known that a systemic inflammation state underlying the gut–liver–brain axis alteration, which includes direct effects of systemic pro-inflammatory molecules in the brain (that can modulate the cerebral effect of ammonia), and recruitment of monocytes after microglial activation, can contribute to HE pathogenesis (Butterworth 2013).

This synergy between inflammation and ammonia probably relates to the effect of the inflammatory mediators and reactive molecules on BBB permeability, the entrance of ammonia and cytokines into the brain, and the consequent microglia activation and neuroinflammation (Butterworth 2013; Aldridge, Tranah, and Shawcross 2015).

As a common clinical finding in cirrhotic patients, muscle loss can also play a role in the pathogenesis of hyperammonemia, since muscles contribute importantly to the extrahepatic ammonia metabolism (Ali and Nagalli 2022).

Many other factors, such as oxidative stress, neurosteroids, increased bile acids, and impaired lactate metabolism likely contribute to the development of HE (Hadjihambi et al. 2019; Liere, Sandhu, and DeMorrow 2017).

Other elements, like cerebrospinal fluid composition, glymphatic flow, cerebral energy metabolism, neurotransmission and cell-cell communication were shown to be altered in HE (Butterworth 2013; Weiss et al. 2016; Hadjihambi et al. 2019; Lu et al. 2019; Bjerring, Gluud, and Larsen 2018).

1.2.2.3. Precipitating factors

There are certain elements or clinical conditions that can precipitate an episode of HE. Some of them are linked to the multiple pathological mechanisms explained in the previous section.

These factors include the presence of gastrointestinal bleeding, infections (especially spontaneous bacterial peritonitis, linked to ascites), constipation, electrolyte imbalances like hyponatremia, alcohol consumption, acute kidney injury, TIPS placement, and use of certain drugs acting on the central nervous system, such as opioids and benzodiazepines (Poudyal et al. 2019; Patidar and Bajaj 2015)

The study of Poudyal et al. (2019) demonstrated that, in a population of 132 patients with OHE, the most common precipitating factor was infection (49.2%), followed by electrolyte imbalance (41%), constipation (33.33%), and gastrointestinal bleeding (16%). Among the infectious causes, spontaneous bacterial peritonitis (18.2%) was the most common precipitant factor, followed by 14.4% respiratory tract infections, 13.7% urinary tract infections and 3% represented by fever of undetermined cause.

Multiple factors contributing to overt hepatic encephalopathy

Beyond the precipitant events previously described, other factors can act simultaneously to sensitize the brain and promote a dysfunctional state (figure 7) (Rose et al. 2020).

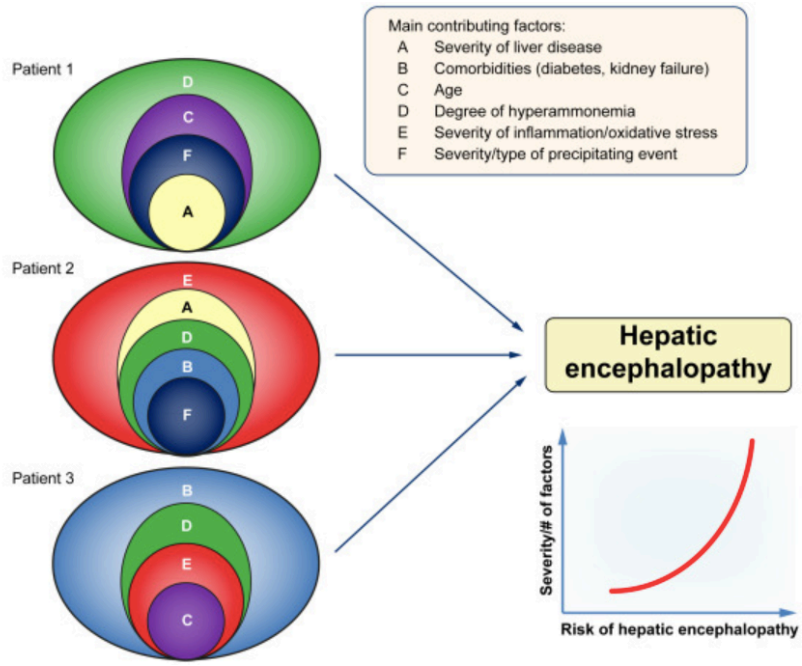


Figure 7. Brain dysfunction in cirrhosis results from the synergistic effect of multiple pathogenic factors. The severity of liver disease, presence of extrahepatic comorbidities, age, levels of ammonia, inflammation and oxidative stress, along with the severity or type of precipitant event can impact the cerebral function and contribute to hepatic encephalopathy. Image from (Rose et al. 2020).

1.2.2.4. Classification and grade

Hepatic encephalopathy is classified into 2 main subdivisions according to the presence of subclinical or clinical manifestations in CHE and OHE, respectively.

As a pre-clinical stage of HE, CHE combines minimal HE (MHE) and HE grade 1. CHE represents the milder expression of the syndrome and is only detectable by specific tests, such as psychometric, electrophysiological, and other functional brain measures (Amodio et al. 2004; McCrea et al. 1996). The tests will be explained in section 1.2.2.5.

CHE is characterized by subtle cognitive and psychomotor alterations, without evident clinical symptoms. It does not include evident disorientation in time and space, and the symptoms might not be recognized as pathologic by the patient or caregiver.

The spectrum of typical neurocognitive alterations in CHE particularly involves the areas of attention, alertness, response inhibition, executive functions, working memory,

psychomotor speed, motor skills and coordination, and visuospatial ability (Agrawal, Umapathy, and Dhiman 2015; Ridola, Cardinale, and Riggio 2018).

OHE (grades II, III and IV) represents the more advanced grades of the syndrome, with the presence of obvious mental changes, including inappropriate behaviour, gross disorientation, confusion, lethargy, asterixis and coma, requiring hospitalization. The classification for OHE grades is based on the West Haven Criteria (table 3).

WHC + MHE	ISHEN	Description	Criteria	Comment
Unimpaired		No encephalopathy	Tested: normal	
Minimal	Covert	• No clinical evidence of mental change • Altered neuropsychometric test, exploring psychomotor speed/ executive functions	Abnormal results of established psychometric tests without clinical manifestations	No universal criteria for diagnosis
Grade I		• Lack of awareness • Euphoria or anxiety • Shortened attention span • Impairment of addition or subtraction • Altered sleep rhythm	Oriented in time and space, but possible presence of some cognitive/ behavioral decay	Clinical findings usually not reproducible
Grade II	Overt	• Lethargy or apathy • Disorientation for time • Obvious personality change • Inappropriate behavior • Asterixis • Dyspraxia	Disoriented for time in 3 of the following: day of the month/ week, months, season, year	Clinical findings variable, but reproducible to some extent
Grade III		• Somnolence to semistupor • Responsive to stimuli • Confused • Gross disorientation • Bizarre behavior	Disoriented also for space in 3 of the following: country, state/region, city or place	Clinical findings reproducible to some extent
Grade IV		• Coma	Absence of response, even to painful stimuli	Comatose state, usually reproducible

Table 3. The West Haven Criteria for hepatic encephalopathy grading. The diagnosis is made in the context of liver dysfunction and/or portosystemic shunting and excluding other neurological diseases. WHC: West haven criteria; MHE: minimal hepatic encephalopathy; ISHEN: International Society for Hepatic Encephalopathy and Nitrogen Metabolism; HE: hepatic encephalopathy. Modified from Vilstrup et al., 2014.

Beyond the grade of severity, HE is also classified according to the underlying disease, its time course, and precipitating factors (Vilstrup et al., 2014) (table 4).

HE type A is due to acute liver failure, type B due to portosystemic shunts or bypass without CLD and type C, due to cirrhosis.

According to the grade of severity, HE is classified as CHE, represented by minimal HE and HE grade 1, and OHE, represented by grades 2, 3 and 4.

HE can be classified as episodic if OHE didn't occur in the previous 6 months, recurrent if a previous episode occurred in the previous 6 months, and persistent if HE symptoms don't resolve completely over time.

HE is classified as precipitated when a factor related to the episode is found, or spontaneous when no precipitant factor can be identified.

Type	Grade		Time Course	Presence of precipitant factor
A Acute liver failure	Minimal 1	Covert HE Psychometric tests	**Episodic** No further HE for ≥ 6 months	**Precipitated** Specific factor found
B Porto- systemic bypass or shunt	2 3	Overt HE West Haven Criteria	**Recurrent** Further episode within 6 months	**Spontaneous** No precipitant factor found
C Cirrhosis	4		**Persistent** Never resolved	

Table 4. Hepatic encephalopathy classification according to underlying disease, grade, time course and presence of precipitant factor. Type shows the underlying condition leading to HE; grade shows a subdivision between the progressive phases; time course divides HE according to its pattern of presentation, and the presence of precipitant factor refers to a causal factor identified in an HE episode. HE: hepatic encephalopathy. Adapted from Vilstrup et al., 2014.

1.2.2.5. Diagnosis of CHE: neuro-psychometric and neurophysiological tests

In the absence of obvious clinical alterations of OHE, neuro-psychometric or neurophysiological tests can be performed to identify the presence of CHE. Despite the presence of multiple tests available, there is still a lack of consensus on what test to use for the

diagnosis. Furthermore, is difficult to find one single test that can access all the multiple cognitive domains impaired in the subclinical phases of HE.

The Psychometric Hepatic Encephalopathy Score (PHES), is considered the current gold standard for diagnosing CHE (Weissenborn et al. 2001), but multiple other tests emerged, aiming to be easier to apply in clinical practice. PHES is frequently used to compare results with new tests, to better define cut-offs. Despite of being the gold standard, PHES can fail to detect the early cognitive changes in about 40% of patients (Gimenez-Garzo et al. 2017). The PHES norms also have to be stablished in each country, and currently have not been established in Canada.

There is a tendency for the development of computerized techniques after the traditional paper-based ones, and tests like Critical Flicker-Frequency (CFF), Continuous Reaction Time (CRT), Inhibitory Control test (ICT) and Scan test were developed, but none of them became widely used in clinical practice.

Stroop EncephalApp was developed in the United States as a promising tool for the diagnosis of CHE and uses a mobile application to perform the evaluation in around 10 minutes. More recently, a short version was tested to be performed in just one minute: the QuickStroop (Acharya et al. 2022).

The main psychometric tests used for the detection of CHE are described as follows:

Psychometric Hepatic Encephalopathy Score

Its diagnosis is validated internationally and includes 5 subtests (figure 8):

- Number Connection Test A (NCT-A): dispersed numbers are supposed to be connected in serial order as quick as possible

- Number Connection Test B (NCT-B): dispersed numbers and letters are supposed to be connected in alternating series as quickly as possible (1-A-2-B..)

- Digit Symbol: digits from 1 to 9 are presented with different symbols on the top of the sheet. The right symbol is to be written under each number until complete all the blank cases

- Serial Dotting: to draw a dot inside each circle as quickly as possible

- Line Tracing: a line is to be traced inside a model line as quickly as possible.

PHES requires trained professionals to apply and interpret the results (Weissenborn et al. 2001; Gabriel et al. 2021).

The diagnosis of CHE is defined by comparing to the norms for each country, matched by age and educational level, and it is expressed as the number of standard deviation (SD) above or below the mean.

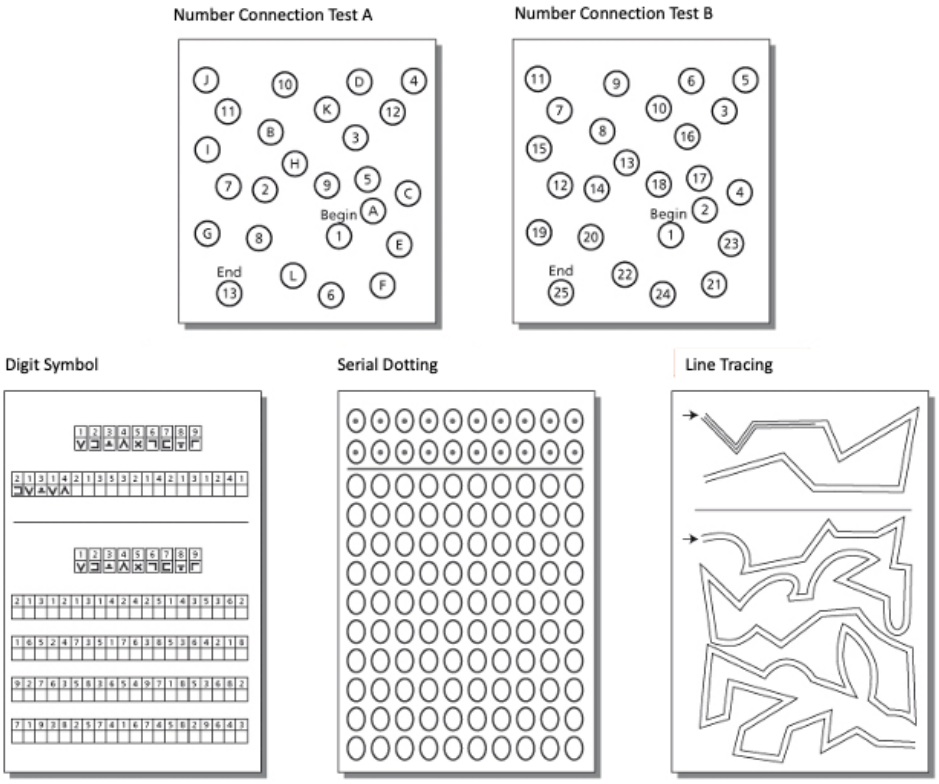


Figure 8. The battery of tests of Psychometric Hepatic Encephalopathy Score. The Number Connection tests A and B, Digit Symbol, Serial Dotting and Line Tracing are the 5 tests performed to produce the PHES score, the gold standard for the diagnosis of covert hepatic encephalopathy. Modified from (Tiberi et al. 2015).

Number Connection Test A and B (NCT-A and B)

These tests are part of the PHES. They are recommended when the norms for PHES are not available.

Repeatable Battery for the Assessment of Neurological Status (RBANS)

It is a paper-pencil test, initially introduced in 1998, designed for the detection of dementia, but also employed to diagnose cognitive impairments in various diseases.

It requires psychologists for interpretation and includes 12 subtests: list learning, story memory, figure copy, line orientation, picture naming, semantic fluency, digit span, coding, list recall, list recognition, story recall and figure recall (Randolph et al. 1998; Mooney et al. 2007).

Clock and Star Drawing tests

The test consists in copying a five-pointed star and a clock face from a template and correlating the results with grades of HE. Constructional apraxia, measured by this test, refers to the inability of patients to copy accurately drawings or three-dimensional constructions and is a well-recognized manifestation of HE (Edwin et al. 2011).

The test offers an option for bedside assessment of HE, but it's not widely used. Many different results might be found, and the diagnosis of CHE is not always clear.

Animal Naming Test (ANT)

The Animal Naming Test is an easy and simple assessment that can be done at the bedside or office for screening of CHE and predicting OHE. It consists in naming as many animals as possible in 1 minute. The test has been shown to predict 1-year risk of OHE and death (Campagna et al. 2017).

SCAN Test

The Scan test is a computerized test for CHE diagnosis and consists in recognizing a digit in pairs in the middle of a series of random numbers displayed for 3 seconds on a computer screen, and pressing digits on the keyboard according to the presence or absence of common digits (figure 9).

The mean reaction times and the percentage of errors are recorded, and the results are evaluated using the reaction times weighted by the number of errors (Amodio et al. 1998; Luo et al. 2020).

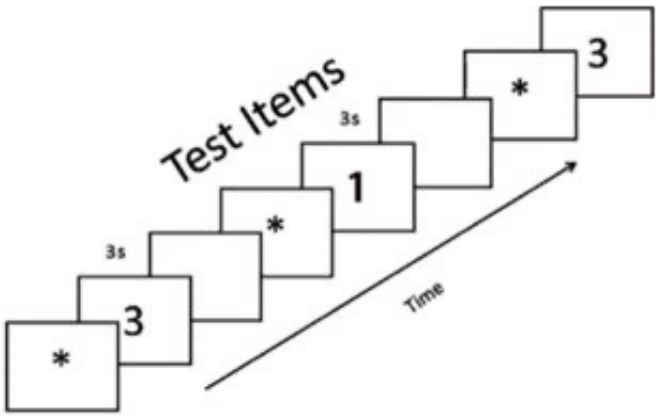


Figure 9. The Scan test. The task consists in pushing the digit on the keyboard according to the identification of a common digit in the pairs of numbers on the screen. Image from (Luo et al. 2020).

Continuous Reaction Time (CRT)

This is a computerized test that requires software, headphones and a trigger button. The activity consists in pressing a button as a response to auditory stimuli. After a 2–minute instruction, sounds are delivered at random intervals from 2 to 6 seconds (beeps at 500 Hz and 80 dB) and the patient is instructed to press the button as soon the beep is heard. The software registers the response times and calculates the CRT index (Lauridsen et al. 2017).

Inhibitory Control Test

It is a computerized test, that presents a continuous stream of letters on a computer screen, and the patient is instructed to hit a button when an X is preceded by a Y or vice-versa. The patient should not respond if an X is followed by an X or a Y is followed by a Y (lures) (figure 10).

This test requires highly functional patients, but demonstrated good validity (Bajaj et al. 2008).

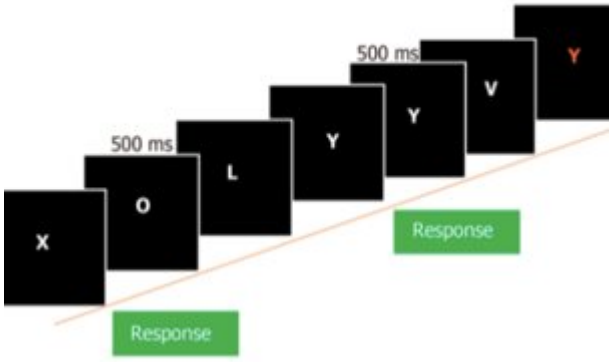


Figure 10. The Inhibitory Control Test. The assessment consists in hitting a button according to the order of appearance of X-Y or Y-X letters. The response should be inhibited if the sequence Y-Y or X-X is identified. Image from (Luo et al 2019).

Critical Flicker-Frequency (CFF)

This test requires specialized equipment, and it was designed originally as an ophthalmological test to measure optical nerve lesions and visual acuity.

Using a headset, the patient is required to press a stop button in response to specific changes in visual stimuli, every time the frequency of the light flickering changes (from 60 Hz downwards).

This test is not valid in persons with red-green color blindness (Kircheis, Hilger, and Haussinger 2014).

Stroop EncephalApp test

This is an application that can be performed on a smartphone or tablet, where the subject is required to choose quickly the color of the word presented on the screen, not the color the word means (figure 11).

This is a rapid and simple test to apply but requires the patient to be familiar with iPhone/iPad (Allampati et al. 2016).

Figure 11. The EncephalApp Stroop Test. The test consists in choosing correctly and rapidly the color corresponding to the color of the hashtag symbol for 5 runs, the phase represented by the Off State (figure in the middle), as well as choosing the color of the word for 5 runs, representing the On State (figure at right). Image from www.encephalapp.com. Website consulted on 16/04/2022.

Quick Stroop

This is the most recent test developed for the diagnosis of CHE. It is a short version of Stroop EncephalApp and includes only 2 runs on the Off State to provide CHE screening in 1 minute, with similar accuracy as the traditional EncephalApp (Acharya et al. 2022) (figure12).

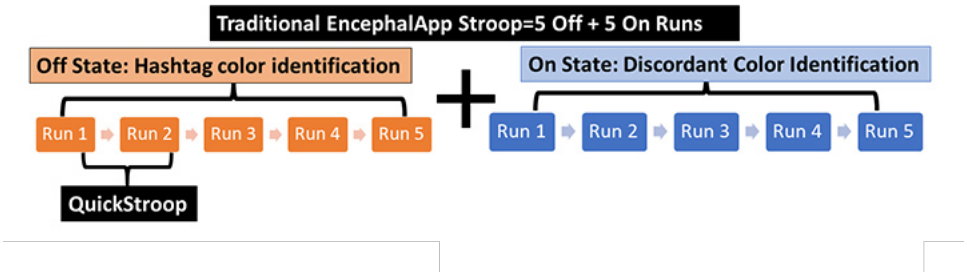


Figure 12. The QuickStroop, a shortened version of the EncephalApp Stroop Test. QuickStroop is composed by 2 runs on Off State, where the subject has to identify the hashtag symbol color, in comparison to the 10 runs on the EncephalApp Stroop (5 runs Off State + 5 runs On State). Image adapted from (Acharya et al. 2022).

Neurophysiological test used for assessment of covert hepatic encephalopathy: the Electroencephalogram (EEG)

The EEG is a neurophysiological test that offers an objective measurement of neuronal activity, that doesn't require the patient's cooperation and doesn't have the risk of a learning effect.

Alterations in the oscillatory characteristics of brain neural networks are found in HE and can be revealed by the EEG (figure 13), but the difficult interpretation can decrease the use as a diagnostic tool. Besides, the results are nonspecific and can be altered by metabolic disturbances (Amodio and Montagnese 2015).

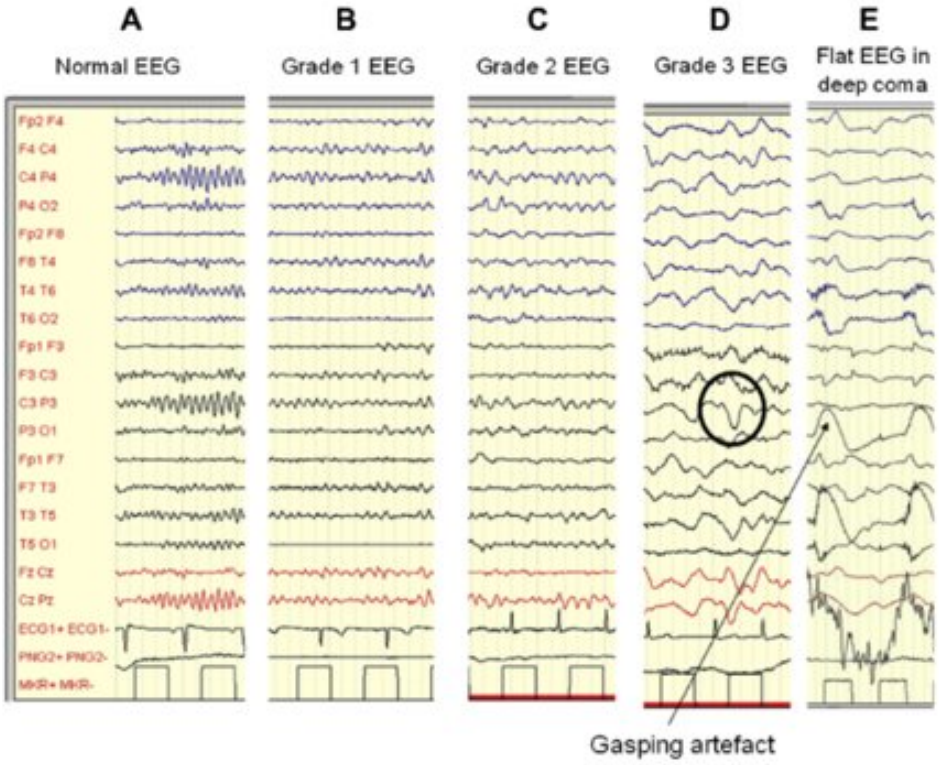


Figure 13. The Electroencephalogram alterations in hepatic encephalopathy. The normal EEG findings (A) are followed by progressive alterations related to HE severity shown in B, C, D and E. Image from (Amodio and Montagnese, 2015).

Despite a large number of tests available, the main challenges regarding the applicability are the need for specialized staff or equipment, the time required to conduct the test, difficulty in interpretation of results and definition of cut-offs applicable widely for all populations, as well as the lack of norms for the tests in multiple countries.

The list of the main tests developed for the diagnosis of CHE and their respective cognitive domains assessed are summarized in table 5.

Tools for diagnosing covert hepatic encephalopathy

Test	Category/ time required	Cognitive domains or functions assessed
PHES[1]	Paper pencil 15-20 minutes	Motor speed, motor accuracy, concentration, (shift) attention, visual perception, visual-spatial orientation, visual construction and memory
NCT-A and NCT-B	Paper pencil 10 minutes	Psychomotor speed, visual-spatial orientation, shift attention
RBANS[2]	Paper pencil 30-35 minutes	Immediate memory, visuospatial/ constructional domains, language, attention and delayed memory.
Clock and star drawing[3]	Paper pencil 3-5 minutes	Constructional apraxia
Animal Naming Test[4]	No equipment 1 minute	Semantic fluency, verbal recall and retrieval, self-monitoring of cognition
Scan test[5]	Computerized 20 minutes	Evaluates working memory, vigilance, and attention
Continuous Reaction time[6]	Computerized 12 minutes	Motor reaction speed, sustained attention, and inhibitory control
Inhibitory Control test[7]	Computerized 15-20 minutes	Attention, working memory and response inhibition
Critical Flicker-Frequency[8]	Specialized equipment 10 -15 minutes	Visual discrimination, attention, general arousal

EEG[9]	Neurophysiological 15 minutes to perform	Neuronal electrical activity
Stroop test-EncephalApp[10]	Mobile phone app 10 minutes	Selective attention, response inhibition, psychomotor speed

Table 5. Psychometric tests used in cirrhotic patients for diagnosis of covert hepatic encephalopathy. PHES: psychometric Hepatic Encephalopathy Score; NCT-A and B: Number connection test A and B; RBANS: Repeatable battery for the assessment of neurological status; EEG: electroencephalogram. 1. (Weissenborn et al. 2001; Gabriel et al. 2021), 2. (Mooney et al. 2007), 3. (Edwin et al., 2010), 4. (Campagna et al. 2017), 5. (Luo et al. 2020), 6. (Lauridsen et al. 2017), 7. (Bajaj et al. 2008), 8. (Kircheis, Hilger, and Haussinger 2014), 9. (Amodio and Montagnese 2015), 10. (Allampati et al. 2016).

1.2.2.6. Treatment options for hepatic encephalopathy

Currently, the guidelines provide instructions for the treatment of patients with OHE, but there is a lack of consensus on the testing and therapy approach to CHE.

The main treatments currently available for HE management are as follows:

a. Nonabsorbable Disaccharides (lactulose or lactitol): considered the first line treatment for OHE. It acts as a laxative that decreases the production and absorption of ammonia in the gut, increasing the fecal nitrogen waste. The side effects might difficult adherence (abdominal cramping, excessive diarrhea and flatulence).

b. Rifaximin: a minimally absorbed oral antibiotic, effective against gram positive, gram negative, aerobic and anaerobic bacteria. It has a direct effect in the gut, reducing the ammonia-producing enteric bacteria. Usually, rifaximin should be used as add-on therapy to lactulose if needed, but the high cost can be impeditive for wide use. Rifaximin is used as secondary prophylaxis and is not considered for the acute treatment of an OHE episode.

c. Branched-Chain Amino Acids (BCAAs): supplementation with BCAA may improve nutrition and revert the loss of muscle cell mass, participating in the detoxification of ammonia. Used as an alternative (or addition) to for patients who didn't respond to the first line therapies (a and b).

d. L-Ornithine-L-Aspartate (LOLA): decreases the ammonia in the blood by stimulation of the urea cycle (which metabolizes ammonia to urea) in the liver, and provides the substrate for

glutamine synthetase, an ammonia-removal enzyme that converts ammonia into glutamine (Kircheis and Luth 2019). Also used as an alternative for first-line therapies for non-responders to lactulose and rifaximin.

e. Probiotics: live bacteria that can improve gut dysbiosis and decrease ammonia production.

f. Nutrition: a normal apport of protein is recommended (1.2–1.5 g protein/kg/day), since malnutrition is very frequent in cirrhosis and is associated with increased risk of HE and poor prognosis.

g. Embolization of portosystemic shunts: alternative for patients who have recurrent episodes or difficult to treat with other therapies.

Other emerging therapies seem promising to improve treatment and include faecal microbiota transplantation (Bajaj et al. 2017), ornithine phenylacetate (Wright et al. 2012, Rahimi et al. 2021) and glycerol/ sodium phenylbutyrate (Weiss et al. 2018) all currently being tested in clinical trials.

Alternatives being developed in the pre-clinical phase include genetically modified *E. coli* (Kurtz et al. 2019), and activated carbon microspheres (Bosoi et al. 2011).

Finally, in the case of CLD and the presence of persistent HE despite the treatment strategies, OLT remains the only curative treatment (Bajaj, 2010; Vilstrup et al., 2014).

Primary Prophylaxis

Currently, there is no consensus on treating patients with CHE. But multiple studies have shown the benefit of primary prophylaxis, which means the use of HE- targeted therapies before the development of the first OHE episode.

Lactulose improved CHE in 66% of patients after 3 months, when compared to controls, by improving results in psychometric tests after treatment (PHES and CFF), and also was effective to prevent a first episode of OHE for one year (Sharma et al. 2012).

Lactulose also could improve health-related quality of life (HRQOL) in patients with CHE (Prasad et al. 2007), measured by the Sickness Impact Profile (SIP).

Probiotics were previously found to be effective in preventing OHE for one year, and improved PHES scores and CFF thresholds after 3 months of treatment (Lunia et al. 2014). However, the studies suggest not all probiotics could offer a benefit. VSL#3, a probiotic mixture that has a therapeutical effect in multiple diseases, was considered a good option, offering results similar as the standard therapy for CHE improvement (Pratap et al. 2015). A meta-analysis of randomized trials, including 1152 patients have found the use of probiotics was effective decreasing hospitalizations, improving CHE and preventing OHE in comparison to placebo, but similar to lactulose, what could offer a therapeutic alternative in patients who don't tolerate lactulose (Saab et al. 2016).

Rifaximin was shown to improve cognitive function and HRQOL in patients with CHE after 8 weeks of treatment when compared to placebo (Sidhu et al. 2011), and driving simulator performance during the same period of treatment (Bajaj et al. 2011).

A meta-analysis including 25 trials found that rifaximin, lactulose, the combination of lactulose and probiotics, LOLA, and probiotics as monotherapy, were effective in reversing CHE compared with placebo (Dhiman et al. 2020).

The results demonstrate that the ammonia-lowering strategies are effective to prevent the first episode of OHE, revert CHE and improve HRQOL in cirrhotic patients, identifying a care gap in the management of patients with cirrhosis.

General principles of management of hepatic encephalopathy

The initial management for an acute episode of OHE is well defined. It consists in providing support care, identifying, and correcting the precipitant factor and starting the use of long-term HE-target therapies to treat the current episode and prevent possible recurrence (secondary prophylaxis). OLT will be discussed and considered (Khungar and Poordad 2012).

These management measures aim to prevent recurrent OHE and decrease the risk of mortality.

The diagnosis of the spectrum of neuropsychiatric abnormalities found in cirrhosis is made after the exclusion of other brain diseases. For this reason, other exams are usually performed to exclude possible differential diagnoses, like computerized tomography (CT) scan and magnetic resonance imaging (MRI).

For HE type C (resulting from cirrhosis), the general principles of management according to the 2014 AASLD-EASL Practice Guideline are shown in table 6.

Hepatic Encephalopathy- General principles of treatment
▪ Primary prophylaxis is not required, unless the patient has a high risk of OHE
▪ All patients with overt HE should be treated
▪ Secondary prophylaxis is required after an episode of OHE
▪ Reference to liver transplantation for patients with liver failure and persistent HE.

Table 6. General principles of treatment for hepatic encephalopathy type C. Recommendations according to the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver (2014 AASLD- EASL). *Not all patients are eligible for liver transplantation, depending on their clinical condition. Adapted from (Vilstrup and al., 2014).

Around 80% of the patients show improvement after correction of precipitant factors (Strauss et al. 1992) and a positive response to the first-line medication can reinforce the diagnosis of HE, especially in the presence of a rapid response. The prolongation of symptoms beyond 72h suggests the further investigation of other causes of mental alterations (Ferenci et al. 2002).

For patients classified in grades 3-4, further measures are needed, such as admission to an intensive care unit and intubation in some cases. The main steps in the management of OHE are summarized in figure 14.

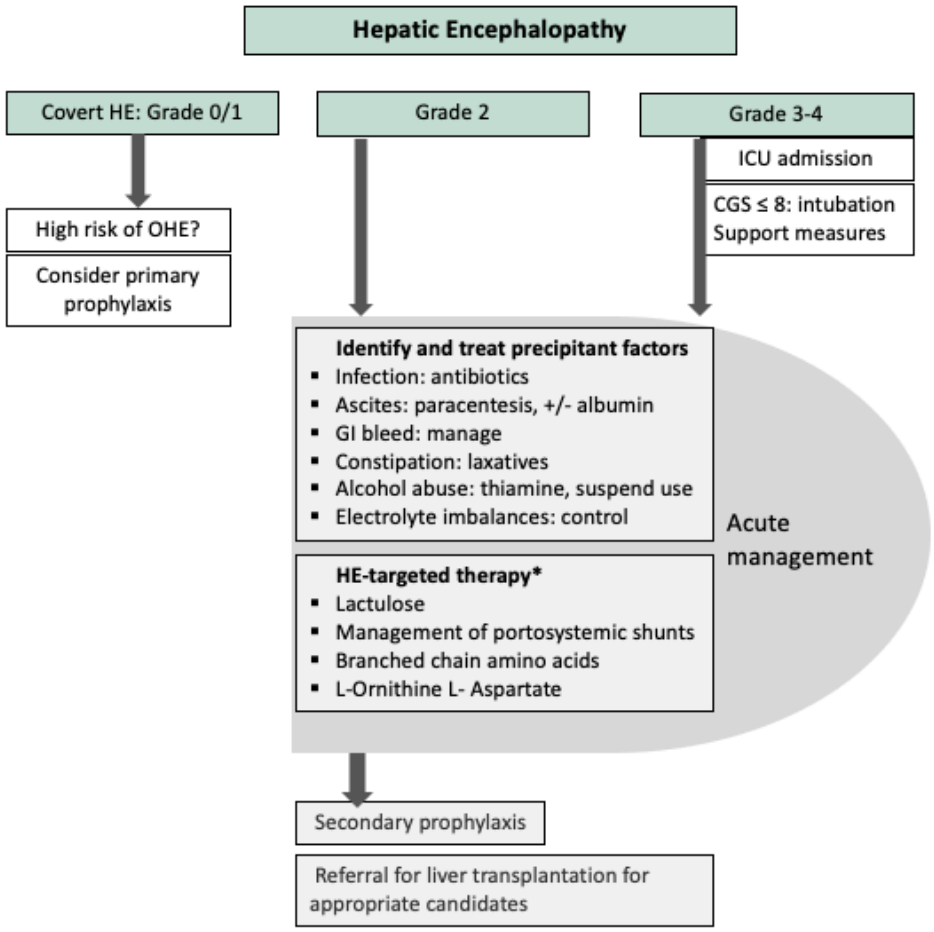


Figure 14. The management of hepatic encephalopathy. Covert HE doesn't necessarily require treatment (grades 0-1), but overt hepatic encephalopathy (grades 2-4) does. Grades 3-4 require further support care due to the severity. *Main HE-targeted therapies currently available. ICU: intensive care unit; CGS: Coma Glasgow Scale; OHE: overt hepatic encephalopathy. Figure adapted from (Rose et al. 2020).

Evaluation of mental status of patients with cirrhosis

The assessment of the neurocognitive functions in cirrhotic patients follows different pathways, according to the presence or lack of evident alterations, is based on different evaluations for diagnosis, can differ in management according to the HE grades and can be associated or not with the presence of precipitant factors.

A summary of the main steps to the evaluation of mental status in cirrhotic patients, classification according to HE grades, and management is demonstrated in figure 15.

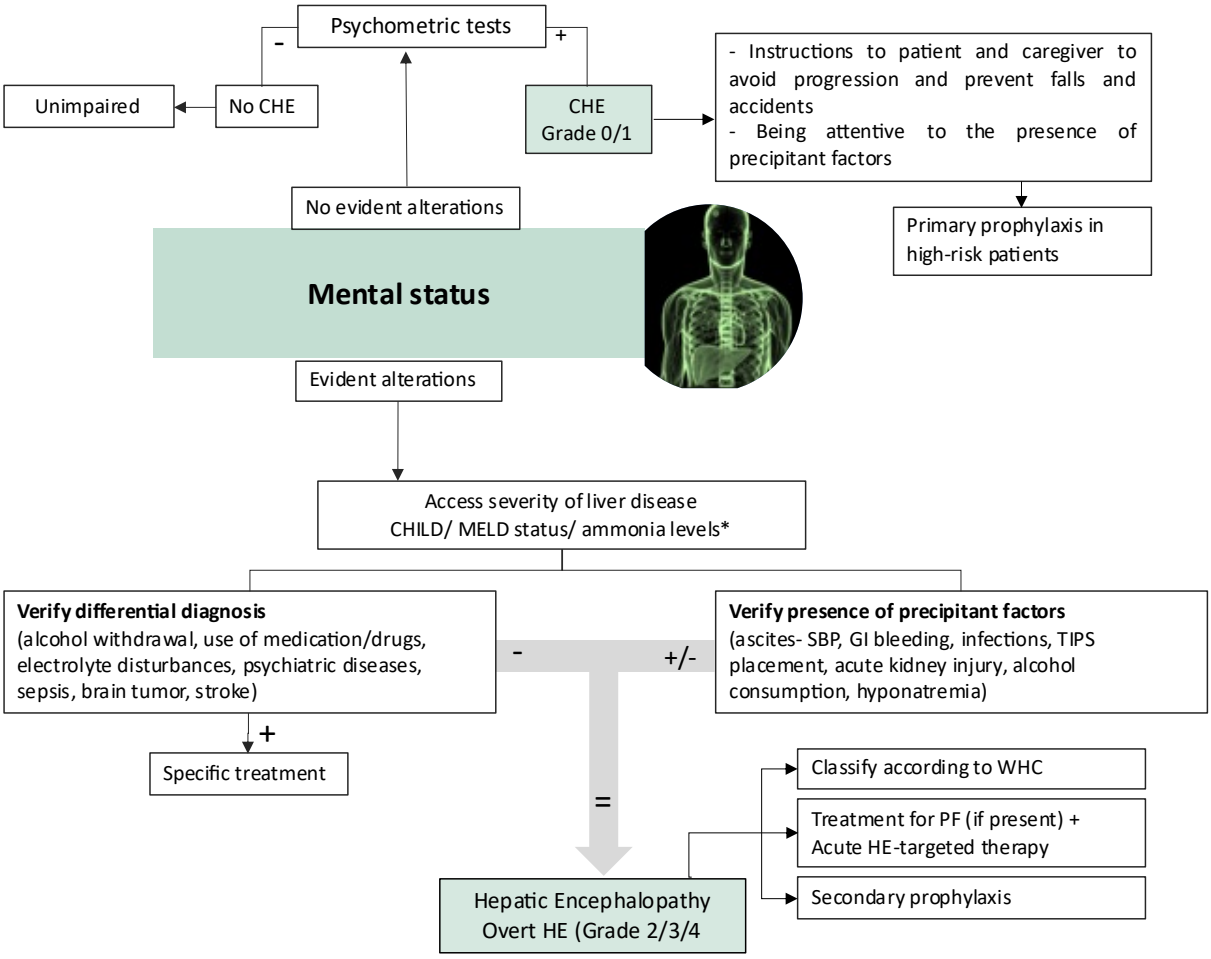


Figure 15. Evaluation of mental status in patients with cirrhosis. The flowchart shows the pathway for the assessment, diagnostic and main aspects of the management of altered mental status in cirrhotic patients. Some differential diagnosis can be confounded as precipitant factors. The resolution of the symptoms with the treatment of differential causes excludes the diagnostic of hepatic encephalopathy; the absence of resolution with the correction of the cause added to the resolution with the HE-targeted therapy confirms the diagnostic. *High ammonia levels are not mandatory for the diagnostic of hepatic encephalopathy, but the normal values might suggest the presence of a differential diagnosis. WHC: West Haven Criteria; HE: hepatic encephalopathy; CHE: covert hepatic encephalopathy; CHILD: Child-Pugh score; MELD: model for end-stage of liver disease; SBP: spontaneous bacterial peritonitis; TIPS: transjugular intrahepatic portosystemic shunts; GI: gastrointestinal; PF: precipitant factors.

1.3. Cambridge brain sciences cognitive assessment

The cognitive tests used in the present study are developed by CBS, which hosts a web-based platform (https://www.cambridgebrainsciences.com) where the tests can be accessed.

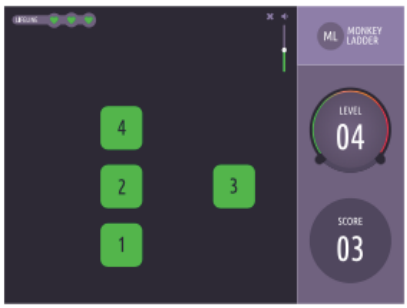
CBS contains a battery of neurocognitive tests, particularly developed for healthcare or research settings, whose tasks have been used by more than 300 studies up to date.

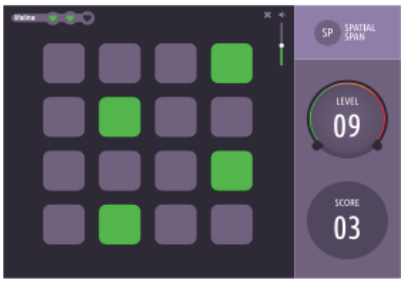
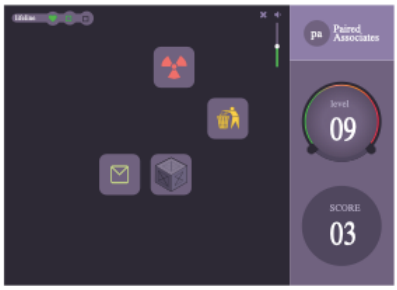
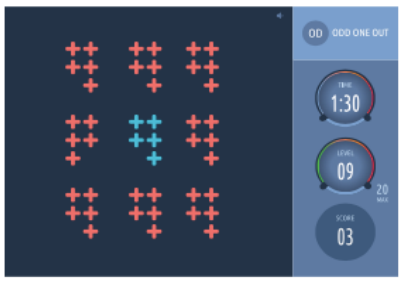
The tests are designed to allow efficient self-assessment and secure data collection and measure different main cognitive spheres.

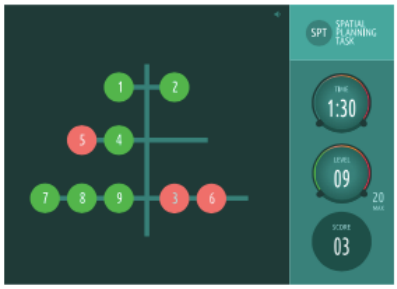
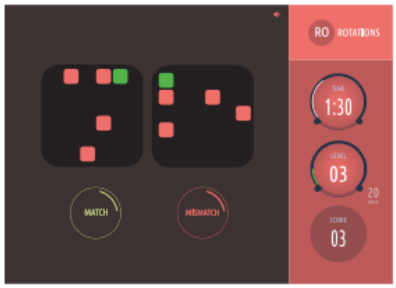
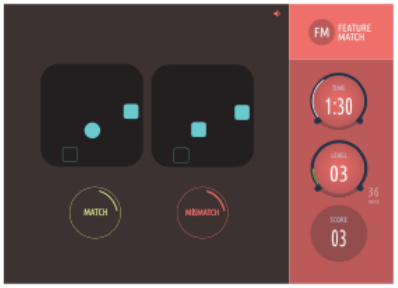
1.3.1. Tests

The complete assessment, which includes a total of 12 tests and lasts approximately 35 to 45 minutes to complete, evaluates multiple abilities related to the main cognitive domains.

The tests administered, the capacities accessed, and the tasks are illustrated in table 7.

 MONKEY LADDER A visuospatial working memory task.	• **Monkey Ladder**: Assesses visual spatial working memory (memorize information and use it according to different circumstances) • Task: to memorize numbers that appear and then disappear on the boxes; to click on the boxes that contain the numbers in numerical order. Right or wrong answers set the number of boxes for the next run

 SPATIAL SPAN A spatial short-term memory task.	• **Spatial Span**: Assesses short-term spatial memory, the ability to temporarily store spatial information • Task: to remember a sequence of flashing boxes that appear one after the other, to click the boxes in the same order he saw them flash after a beep. Right or wrong answers set the number of boxes for the next run
 TOKEN SEARCH A working memory task.	• **Token Search**: Assesses the ability to hold and manipulate information in spatial working memory • Task: to click on the boxes until find a token. When it disappears, continue to click on other boxes to find other tokens without clicking where previous tokens were found
 PAIRED ASSOCIATES An episodic memory task.	• **Paired Associates**: Assesses episodic memory, which is the ability to remember specific events, associated with the context in which they occurred • Task: To remember which object appeared in which box previously shown, one after the other. Then click on the respective box, according to the object shown on the middle of screen.
 ODD ONE OUT A deductive reasoning task.	• **Odd One Out**: Assesses the ability to apply rules to information in order to arrive at a logical conclusion • Task: to identify if a series of shapes is different from others. Shapes and colors are added in each run, making the identification consider multiple pieces of information.

 **SPATIAL PLANNING** A planning task.	• **Spatial Planning**: Assesses the ability to plain ahead, acting with foresight and sequence behavior to achieve specific goals • Task: to rearrange the balls on the frame in numerical order, making as few movements as possible. Only the balls on the extremities can move to empty spaces.
 **POLYGONS** A visuospatial processing task.	• **Polygons**: Assesses the ability to effectively process and interpret visual information, such as complex visual stimuli and relationships between objects • Task: to identify if there are two identical shapes in the screen in middle of other shapes displayed in two panels, by clicking on “match” or “mismatch”. Right answers increase the difficulty.
 **ROTATIONS** A mental rotation task.	• **Rotations**: Assesses the ability to effectively manipulate mental representations of objects in order to draw valid conclusions • Task: to identify if the two panels are identical, when one of them is rotated. Right answers increase the difficulty.
 **FEATURE MATCH** An attention task.	• **Feature Match**: Assesses the ability to use mental focus to monitor a specific stimulus or difference • Task: click “match” or “mismatch” according to the complex array of abstract shapes displayed in two boxes

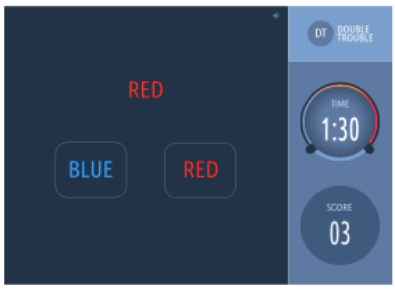
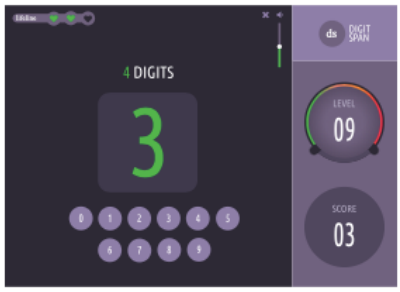
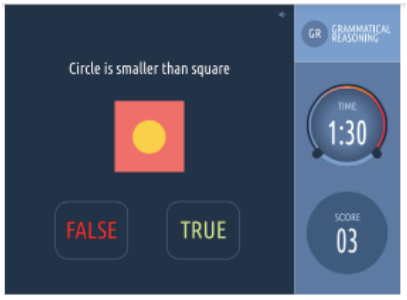
 DOUBLE TROUBLE A Stroop task to assess response inhibition.	• **Double Trouble**: response inhibition test similar to Stroop, that measures the ability to focus on relevant information in order to provide a correct response despite interference or distracting information • Task: to click on the word at the bottom of the screen that correctly describes the color of the word at the top
 DIGIT SPAN A classic verbal short-term memory task.	• **Digit Spam**: assesses short-term verbal memory, the ability to temporarily store information • Task: to click on the numbers on the bottom of the screen in the same sequence as they were displayed before, one by one. Correct answers increase the numbers, wrong answers decrease one digit the next run
 GRAMMATICAL REASONING A verbal reasoning task.	• **Grammatical Reasoning**: measures the ability to quickly understand and draw valid conclusions about concepts expressed in words • Task: to click “True” or “False” to indicate whether the sentence on top of the screen accurately describes the relationship between the shapes displayed • Wrong answers are subtracted from the score

Table 7. Cambridge Brain Sciences cognitive assessment. The 12 tests battery used in this study, assessing simultaneously multiple cognitive domains.

1.3.2. CBS Norms

The CBS health normative database comes from a worldwide sample and was collected from a public study involving over 44,000 participants included (Hampshire et al. 2012), that initially aimed to better understand human intellectual ability. The participants answered a demographic questionnaire and took the full battery of 12 tests. Over than 85,000 volunteers contributed to the CBS health database (cambridgebrainsciences.com).

The CBS norms are divided into blocks of age, gender, and level of education. Each participant from our study who perform the tasks will have their scores classified according to these categories.

The categories representing age are: 18-24, 25-34, 35-44, 45-54, 55-64, 65-74, and 75+. The classification by levels of education include: some high school (or less), high school graduate, some college, college graduate and higher education. The genders are masculine, feminine, and omitted (compared to the entire database). There is no classification according to the language in which the test was performed.

The norms included participants from multiple continents: Africa: 1.5%; North America: 40.8%, South America: 2.7%, Asia: 5.8%, Europe: 39.5% and Oceania: 9.7%. Most participants were from United States, United Kingdom and Canada (cambridgebrainsciences.com).

Chapter 2. Problematic and research objectives

The traditional psychometric tests used in patients with cirrhosis were developed from paper and pencil tests to faster-computerized models. Despite the decrease in the time needed to perform multiple tests from the paper models, the computerized tests may not allow the identification of all alterations in cognitive function at early stages, in comparison to tests that evaluate multiple cognitive domains.

The discrepancy in the diagnosis of CHE and OHE risk prediction is common when using multiple traditional tests simultaneously, which has been demonstrated by multiple previous studies (Gimenez-Garzo et al. 2017; Allampati et al. 2016).

It is important to better understand the pattern of cognitive impairment in the early stages and how they impact the patient with CLD.

The cognitive function is an important predictor of negative outcomes in cirrhosis, and its proper identification is an important step to create efficient predictive models for OHE.

This study aims to characterize in more detail the cognitive profile of patients with CLD before the development of OHE, evaluating different cognitive sub-domains with the CBS battery, a web-based cognitive assessment.

Further objectives include the prediction of the first OHE episode during one year of follow-up using the test scores and identifying if there is a single test out of the 12 which could predict the first hospital admission due to OHE.

Chapter 3. Methods

3.1. Design of the study

The design of this research project is a single-center, longitudinal prospective study, with one year of follow-up.

At the baseline, the most recent laboratory exams were collected retrospectively from the medical records, and the cognitive assessment was performed online.

Patients received phone calls and had their medical records verified each 3 months up to 12 months to verify liver-related and/or HE-related hospitalizations, as to report the presence of triggering events, changes in symptoms, medication and events occurring outside the CHUM.

The follow-up will end 1 year after the cognitive assessment or after the development of an HE episode, OLT or death (figure 16).

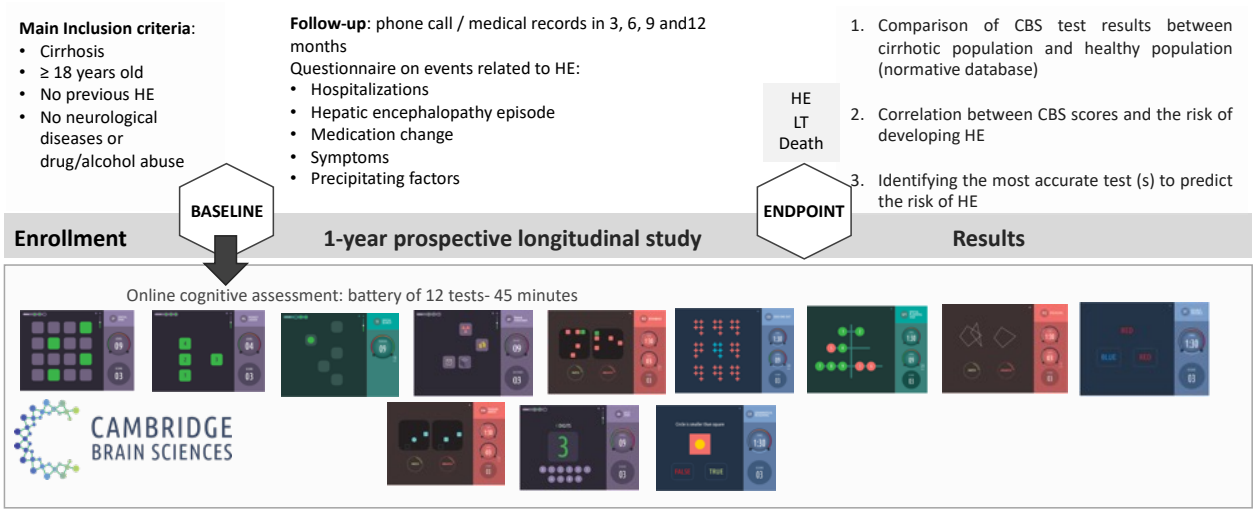


Figure 16. Design of the study. After the inclusion, patients perform the CBS cognitive assessment online and their most recent exams are collected retrospectively from medical records. Follow-up calls are performed 4 times until 1 year after the tests, or until the development of hepatic encephalopathy, the occurrence of liver transplantation on death.

3.2. Ethics

The study protocol was approved by the CRCHUM ethics committee (Annex 1) and all participants received an information and consent form, which they signed before the beginning of the study (Annex 2).

With respect to confidentiality, the data collected is only accessible to the research team and is stored on a secure CHUM/CRCHUM server and via the Redcap platform (version 12.2.4). The results can be disseminated in the form of student reports, scientific publications or congresses, but the participants are not identified in any way during the dissemination of the results.

3.3. Study Population

The experimental approach involves the recruitment of patients with cirrhosis without a history of OHE, followed at the department of Hepatology, from the Centre Hospitalier de l'Université de Montréal (CHUM).

3.4. Recruitment

For the recruitment process, we used the platform Oacis, where the patients' medical records at CHUM could be assessed for a pre-selection (phase I).

From December 2020 to October 2021, we identified patients within the different lists; outpatient clinics, previous hospitalizations, liver transplant waiting list, endoscopy and day clinic (where patients go for same-day paracentesis).

To facilitate the enrollment of participants, in September 2021, we sent hepatologists a weekly email with information on eligible patients whom they would be seeing in the clinic the following week. The hepatologist would then discuss our study with the patient and suggest them to contact us or we would follow up by telephone. This strategy slightly increased the recruited number of patients, impacted by the pandemic restrictions.

The medical records were analyzed according to our inclusion/exclusion criteria to identify eligibility.

3.4.1. Inclusion criteria

We selected participants with the following criteria:

1. Both sexes, 18 years of age and older

2. Having an established diagnosis of cirrhosis by biopsy, Fibroscan or CT-scan

3. Having recent exams in their medical records (no older than 6 months before the enrollment)

4. Able to speak French or English, since our tests and questionnaires were available in both languages

5. Internet access with sufficient familiarity to use and no physical impediment to use it (visual or motor deficiency)

6. Being able to give informed consent.

3.4.2. Exclusion criteria

To be included, the participant could not present one of the following:

1. Previous or current episode of OHE

2. Presence of neurological diseases

3. Currently undergoing continuous therapy with psychoactive drugs: benzodiazepines, anxiolytics, hypnotics, or any medication capable of causing altered mental status during the tests

4. Use of illicit drugs in the past year

5. Past or present alcohol abuse by psychiatric definition: history of alcohol use that results in one or more of the following in the past 12 months: i. Major inability to fulfill responsibilities at work, school or at home ii. Drink in physically hazardous situations, such as while driving a car or operating heavy machinery iii. Have alcohol-related legal problems, such as being stopped while driving under the influence of alcohol or for injuring someone while intoxicated iv. Continue to drink despite relationship problems caused or made worse by excessive alcohol consumption

6. Have a history of head trauma resulting in loss of consciousness for more than 30 minutes.

3.4.3. Participant inclusion process

3.4.3.1. Pre-selection and invitation letter (recruitment phase I)

Patients who fulfilled the criteria, referred or not by hepatologists, received an invitation letter (Annex 3) and a flyer of the project (Annex 4) by mail. In this letter, they received a brief explanation about the research project and a link to the Hepato-Neuro lab website (https://hepato-neuro.ca), so the patient could access more information online (Annex 5). The letter explains that a second contact by phone will be made by our team approximately two weeks after the letter was sent to verify their interest to participate and, if applicable, to confirm the eligibility criteria.

3.4.3.2 Phone call (recruitment phase II)

After sending the letter, we contacted the patient by phone (recruitment phase II). If the patient doesn't answer, a voice message is left saying the patient will be contacted again in 3 to 4 days to verify their interest to participate in the research project explained in the letter they received. At the end of the message, we provide our contact/phone number at CRCHUM in case the patient wants to contact us directly or leave a message.

If the patient doesn't answer the second phone call, no message is left, and one final call is made 4 days later. If they can't be reached by phone after this process, they will not be contacted anymore.

If the patient answers the phone call and wishes to participate, we verify all the eligibility criteria, by asking questions to the patient. If they fit the criteria, all the main information about the project is explained, which includes: 1) a description of the main goals and steps of the project, 2) an explanation about how to access and perform the CBS tests online, 3) the trimestral follow-up calls, and 4) an explanation about how to access and fill the information and consent form, confidentiality criteria, voluntary participation and right to withdrawal.

Using RedCap, we created the database for the project, and multiple automatic emails are sent from this platform, according to the participant's phase.

3.4.3.3 Getting the information and consent form (recruitment phase III)

After filling in the information regarding the eligibility criteria and explaining the project during the phone call, we confirm the patient's electronic address and explain that the first email (Annex 6) would contain the link to access the information and consent form, which should be filled and signed online (recruitment phase III). In the absence of a signed consent after 20 days, the patient receives an email from RedCap to remind them that, if they want to participate, it is necessary to provide the signed consent online (Annex 7). If the patient didn't answer or sign the consent, they are not included in the study.

If the patient signs the form, the patient receives a confirmation message at the end (Annex 8) containing a link to the calendar of the project, hosted by calendly.com (Annex 9), where the patient can choose, during a period of 2 weeks, the day and time they prefer to perform the CBS tests online.

They also receive a confirmation by email, with a copy of their information and consent form (Annex 10). This email also contains the link to access the calendar to schedule the CBS tests, in case they had not done so already.

3.5. Data collection

3.5.1. Medical records

During the pre-selection phase, the medical records were consulted on Oacis, the electronic medical record system used at CHUM, to verify if the patients fit the criteria to be contacted.

If the patient agrees to participate during the next steps, all the information from their medical records required to participate is consulted and stored in our database, which includes demographic data, laboratory results, diagnostic exams for cirrhosis, presence of previous decompensation events, complications and etiology of cirrhosis, medication in use (related to cirrhosis) and hospitalizations.

The exams consulted included liver biopsy, Fibroscan, ultrasonography, CT-scans, MRI, endoscopy and others to verify the diagnostic, presence of decompensating factors and severity of the liver disease.

The biochemistry panel was accessed to demonstrate pertinent exams related to liver disease and to calculate the Child-Pugh and MELD-Na scores. These exams include hemoglobin, platelets, AST, ALT, total bilirubin, albumin, creatinine, sodium and INR. The measurement units and reference values for each test used by CHUM, as well as their meaning, are explained in table 8.

Test	Reference	Characteristics
Hemoglobin (g/L)	130-170	•Protein in the blood that carries oxygen and carbon dioxide to exchange between lungs and tissues in the body •In cirrhosis, Hb levels can be reduced due to multifactorial reasons, like portal hypertension induced sequestration, alterations in erythropoietin. increased blood loss by variceal bleeding[1]
Platelet count x 10^9 /L	130-400	•Also called thrombocytes, they help blood clot and stop bleeding. •Cirrhosis can cause thrombocytopenia by platelet sequestration in the spleen and decreased production of TPO in the liver[2]
AST (U/L)	13- 39	•AST and ALT are liver transaminases that can reflect the activity and destruction of liver cells •Elevated AST levels can be found in liver or muscle damage
ALT (U/L)	10-39	•Enzyme that convert proteins into energy to the liver cells •Increased levels indicate a liver damage
Total bilirubin (µmol/L)	7-23	•Product of the normal breakdown of red blood cells, bilirubin is a yellow pigment that passes through the liver to be

		metabolized prior to excretion in feces and urine. High levels of bilirubin cause jaundice.
Albumin (g/L)	36-45	•One of the main proteins of the body, is produced by the liver and has multiple functions. •It is a marker of liver function, and low levels might reflect liver failure/ poor nutritional status
Creatinine (µmol/L)	53-112	•Creatinine is a breakdown product from muscle and protein metabolism, and reflects the renal function •Cirrhosis can lead to hepatorenal syndrome and progressive levels of creatinine, reflecting decreased glomerular filtration rates
Sodium (µmol/L)	135-145	•Sodium (Na) is an electrolyte in the body that regulates the water balance in and outside the cells, and participate on the neurotransmission •Hyponatremia (low levels of Na) is associated with severity of cirrhosis and can lead to further decompensation
INR	0.8-1.15	•Test that reflects the coagulation time and liver function. •The liver produces the majority of coagulation proteins, reduced in liver injury.

Table 8. Laboratory tests used at the baseline, their respective reference values and characteristics. TPO: Thrombopoietin is a glycoprotein hormone produced by the liver and kidney which regulates the production of platelets. 1. (Privitera and Meli 2016), 2. (Hayashi et al. 2014).

The Child-Pugh and MELD were calculated using the patient's results for all the clinical and laboratory data found on the most recent patient's medical records.

The Child-Pugh score was calculated based on the same scoring system exposed in table 1 at https://www.mdcalc.com/child-pugh-score-cirrhosis-mortality. To calculate the CHILD score, it's necessary to access the presence and grade of HE. Since none of our patients had OHE

(nor a history of OHE), we defined all patients as “no encephalopathy”, or grade 0 for that category. The presence of MHE is not considered for the calculation of Child-Pugh score.

We calculated the MELD-Na score using the website https://www.mdcalc.com/meldna-meld-na-score-liver-cirrhosis. The following formulas were used to convert the biochemical data collected in the file for the calculation of the MELD-Na score:

MELD Score = (0.957 * ln(Serum Creatinine) + 0.378 * ln(Serum Bilirubin) + 1.120 * ln(INR) + 0.643) * 10

MELD-Na = MELD Score - Na - 0.025 x MELD x (140-Na) + 140

Medical records were also consulted to confirm information obtained from the patient during follow-up and to keep the database updated about changes in medication related to cirrhosis, liver-related complications and hospitalizations.

3.5.2. CBS online cognitive assessment

On the day and time chosen by the patient to perform the cognitive tests online, we inserted their information on the CBS website, including the patient’s ID, age, sex, level of education and email to generate a link for that specific patient.

We sent an automatic email using RedCap prior to the scheduled time with the instructions to perform the tests, the link to access the online platform using their own email and a password provided by us (Annex 11). The access to the CBS platform was offered in English and French, according to the patients’ preference. That includes all the interactive pages and the tutorials to perform the tests until it’s concluded.

At the scheduled time, the patient receives a phone call, to make sure they understood the instructions, are capable to connect to the platform and start the interactive tutorials. Phase IV of participation is achieved when the patient performed the CBS tests.

The platform provides itself the instructions on the screen about the next steps until the end. At this point, we leave the patient alone, so they can concentrate and perform the battery of cognitive tests, which takes approximately 45 minutes to be completed. Before all the tests start, the platform offers a training phase with interactive tutorials (figure 17).

Figure 17. CBS interactive tutorials during the training phase. The tutorials allow the participant to navigate with simple instructions on the top of the screen, providing the information so they can perform the tasks. Test name: Double Trouble (the CBS version of the Stroop test).

On the top of the screen, there are simple instructions to interact with the screen, with arrows that allow the participant to go back and forth, read the instructions and perform the tests again if necessary. When they are ready, they choose to finish the tutorial and start the test.

Considering the time necessary to perform the complete battery (35-45 minutes), previous studies observed that patients could present fatigue during the test execution (Honarmand et al. 2019). For that reason, we allowed patients in the present study to have a pause when they reach 50% of the tasks, by receiving a message on their screen.

We remain available during the entire period of the test so the patient can contact us by email or phone call if they need some technical support, but the tests must be performed by themselves without any added help.

If they don't perform the tests within 7 days, RedCap sends an email notification (Annex 12), to remind them to complete the battery by accessing the same link. If they don't complete it, they are excluded from the study.

When they finish the battery of 12 tests, they receive a message saying that they have completed the task and we will receive the results directly on our CBS account (Annex 13).

RedCap also sends an email to confirm we received the results and provide instructions for the next step, which is the first follow-up call in 3 months.

3.6. Follow-up

The follow-up consists of phone calls every 3 months to verify the occurrence of the OHE, liver-related hospitalizations, presence of symptoms or subtle cognitive alterations related to HE, precipitant factors, other causes of hospitalization and changes in medication.

Before calling the patient, we send an email to inform the patient they will receive a phone call in a few days regarding his participation in the research project (Annex 14). If they are not available, they can communicate with us and provide his availability.

During the phone call, we apply a questionnaire that takes from 5 to 10 minutes, depending on the case and the presence of positive outcomes during the last 3 months (Annex 15).

If during follow-up, the patient did not develop an episode of OHE, the follow-up continues every 3 months until 12 months after they performed the online cognitive assessment.

If the patients do develop an episode of OHE, underwent an OLT, died or reached 12 months of follow-up, their participation ends, and they receive a confirmation by phone and email (Annex 16).

3.7. Data analysis

Data was imported from the RedCap database and from CBS reports into SPSS statistics.

CBS test results were expressed as scores, raw scores and percentiles, valid or invalid. The platform can detect if the result is invalid during the test performance by the presence of repetitive clicks on the same place, excessive time without interaction or lack of reasonable answers, for example.

The coefficient of reliability for the CBS battery was assessed using Cronbach's Alpha. Values > 0.70 defined reliability.

The normative scores are standardized so the mean of the healthy population for all scores is represented by zero (Z-scores), and the standard deviation (SD) is equal to 1. This means that if the Z-score of our participants in any test is above zero, it is above the healthy population mean. If the Z-score is negative, it means that it is below the healthy population mean, represented by the normative database.

The Z-scores of all 12 tests were calculated using the raw score, norm mean and standard SD, and compared to the CBS normative database.

The Z-scores for each test of the battery per patient were calculated using the following formula:

$$\text{CBS test Z-score} = \frac{(\text{test raw score} - \text{test norm mean})}{\text{test norm SD}}$$

The individual Z-scores were used to calculate the mean Z-scores and SD of the participants of the study, and they were matched by age, gender, and educational level with the CBS norms.

Composite scores, divided by cognitive domains, were calculated by multiplying the individual Z-score of each of all 12 tests (T1-T12) by the respective factor loading (FL), a value that reflects the contribution of that test to each cognitive domain (short-term memory, reasoning and verbal ability), as defined by a previous study (Wild et al. 2018).

The sum of the 12 values obtained was then divided by the number of tests (Tn= 12) to generate the composite score:

Composite score : ((T1 Z-score*FL) +… (T12 Z-score*FL) / Tn

The cognitive domains were divided into reasoning skills, short-term memory, and verbal processing. All 12 tests contribute to each cognitive domain, according to their component weights.

Missing scores (invalid results) were excluded from the calculations and replaced by the patients' mean for that test. Data imputation was only used to compare domain scores, calculated among patients who had all the 12 test scores.

We also calculated abbreviated Z-scores that include a short version of the CBS battery, using the results of 6 tests instead of 12, as defined by (Honarmand et al. 2019). Based on this previous study, the chosen tests for the abbreviated scores are those that most strongly reflect their respective cognitive domain: Odd One Out and Rotations (reasoning skills), Paired Associates and Monkey Ladder (short-term memory) and Digit Span and Grammatical Reasoning (verbal processing).

Demographic and clinical variables were demonstrated using descriptive statistics, expressed as mean, maximum and minimum values for continuous variables, and frequency and percentage for categorical variables.

One-sample t-test analyzes were performed to compare the Z-scores between our participants and the normative database.

Pearson and Spearman's correlations were used to evaluate the relationship between multiple variables of the study (parametric and non-parametric).

Linear regression models were used to predict the risk of hepatic encephalopathy using the CBS scores.

The p-value threshold for statistical significance was fixed at 0.05.

The analysis was performed in SPSS Statistics (version 27). Figures were created on Prism 9 (version 9.3.1) and Microsoft Excel (version 16.58).

Chapter 4. Results

Thirty-four cirrhotic patients from CHUM were recruited from January to October 2021.

For the recruitment, more than 1500 medical records were accessed during the pre-selection (phase I), and 242 eligible patients were contacted by letter and phone in phase II.

In total, 184 patients answered the phone call, and 104 accepted to answer the recruitment questionnaire and verify the conditions to participate. 80 patients mentioned they were not interested.

Among those who answered the questions, more than 70% reported having access to a computer/tablet/internet and were comfortable using it. Among the 184 patients reachable by phone, 20 patients (10.8%) mentioned spontaneously they were not interested in participating in this model of study, using online assessments, and performing tests at distance.

The main causes for not including patients in the study during phases I and II were: other diagnostics than cirrhosis during the pre-selection on the medical records, patients did not fulfill inclusion/exclusion criteria, were non-reachable by phone or were not interested in participating (figures 18 and 19).

Sixty-six patients accepted to participate in the phone interview, but due to lack of signed consent form (n=24) and other exclusion criteria (n=3), related to computer or internet issues (didn't get access to a borrowed computer or technical problems with their equipment), 27 were excluded (phase III).

Approximately 90% of the patients contacted on the first phone call to verify interest and eligibility were francophone, and approximately 9% were anglophone. Just 1% of patients contacted did not speak English or French.

All patients who accepted to participate in this study spoke French, but 3 of them choose to perform the online assessment in English.

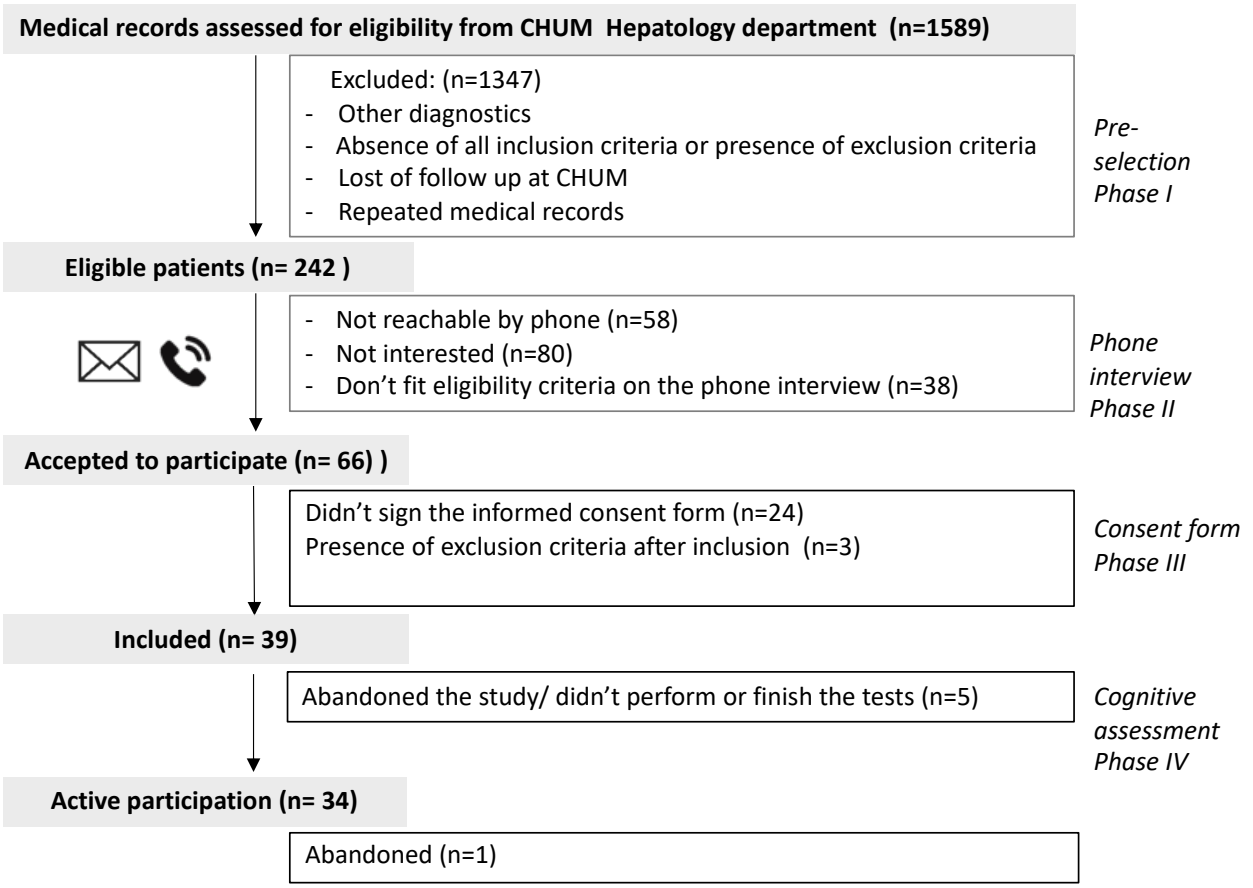


Figure 18. Flowchart for the recruitment of participants.

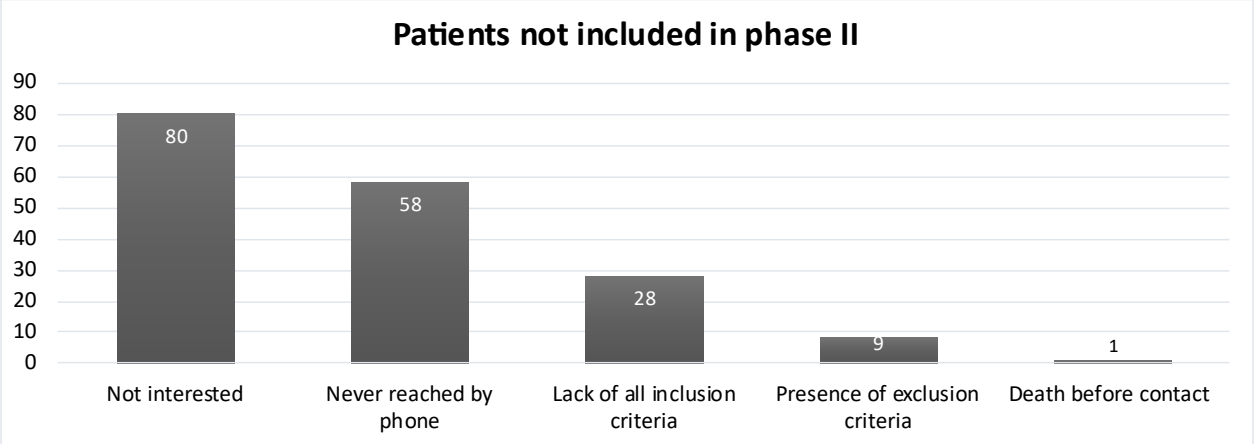


Figure 19. Main reasons for not including patients in the study during phase II. During the phone interview, the figure shows the reasons why patients were not included in the study. The inclusion criteria verified during phone interview include computer/internet access and the exclusion criteria include the presence of visual impairments, abuse of substances or history of head trauma or other neuropathologies.

4.1. Characteristics of the study population

The demographic and clinic characteristics of the participants are presented in table 9. The population of the study was represented by a majority of males (n=21, 61.7%), with a mean age of 60±8.5 years. The main ethnic origin was North America (from Canada). The most prevalent etiologies for liver cirrhosis were viral hepatitis, ALD and NASH.

According to the severity of the liver disease, they were classified as Child-Pugh A (n=26) and B (n=8), with a mean MELD-Na score of 9.

The laboratory exams of the participants showed increased mean values for AST/ALT in comparison to the normal reference values, and total bilirubin mean values were above upper limit of normality.

Seven patients were considered previously decompensated, defined by the presence of ascites, gastrointestinal bleeding and/or hepatorenal syndrome.

Approximately 70 % of the participants had signs of portal hypertension, identified by the presence of ascites, varices and hypersplenism.

Six patients were using antidepressants, but they didn't refer any alterations in their cognition or impediment to participating in the study when asked.

Characteristics of the study population (n=34)			
Sex		**Laboratory exams**	
Male (n/%)	21 (61,7%)	**Hemoglobin (g/L)**	132 (78 – 167)
Female (n/%)	13 (38,2%)	**Platelets (n/L)**	154 (74 – 464)
Age (years)	60.7 (35 - 74.3)	**AST (U/L)**	46 (12-228)
Ethnicity (n/%)		**ALT (U/L)**	42 (8 -261)
North America	28 (82%)	**Total bilirubin (µmol/L)**	23 (5- 119)
Asia	2 (5.8%)	**Albumin (g/L)**	38 (26- 47)
Other	3 (8.8%)	**Creatinine (µmol/L)**	68 (32- 129)
Education (n)		**Sodium (µmol/L)**	139 (133- 145)
High school	6 (17%)	**INR**	1.12 (0.87- 1.64)
College	11 (32%)	**Complications (n/%)**	
University	17 (50%)	**Ascites**	7 (20%)
Etiologies of cirrhosis (n/%)		**GI Varices**	16 (47%)
Viral hepatitis (B,C,D)	8 (23%)	**GI bleeding**	1 (2.9%)
Alcoholic	8 (23%)	**Hypersplenism**	14 (41%)
NASH	12 (35%)	**Presence of HCC**	9 (26.4%)
Autoimmune hepatitis	4 (11.7%)	**Decompensated**	7 (20%)
Primary biliary cholangitis	4 (11.7%)	**CBS Tests version (n/%)**	
Others	5 (14.7%)	**French**	31 (91%)
CHILD (A-B) (n)(%)	26 (82%)- 8 (18%)	**English**	3 (9%)
MELD-Na (value)	9 (6- 19)	**Use of antidepressants (n)**	6 (17.6%)

Table 9. Baseline characteristics of the study population. Some patients might present more than 1 etiology of cirrhosis, as well as multiple complications simultaneously. Age, MELD and laboratory values are presented as mean, minimum and maximum values. NASH: Non-Alcoholic Steatohepatitis, MELD-Na: Model for End-Stage of Liver Disease (sodium), AST: aspartate aminotransferase, ALT: alanine aminotransferase, INR: International Normalized Ratio, GI: gastrointestinal, HCC: Hepatocellular carcinoma, CBS: Cambridge Brain Sciences.

4.2. Cognitive performance among cirrhotic patients

The CBS neuro-psychometric tests were used to investigate the cognitive performance in patients with CLD. The reliability of the battery was assessed using the Cronbach Alpha coefficient (table 10).

Component	Items (n)	Alpha (α)
CBS Battery	12	.74

Table 10. Reliability statistics. Correspond to the measure of internal consistency of the constructs in the study. Construct reliability was assessed using Cronbach's Alpha. Values >.70 define reliability.

The CBS platform reports the validity of the patient's score in each of 12 tests, identifying unusual interactions during tests and classifying them as invalid, therefore being removed from the analysis. The number of valid tests per patient is demonstrated in table 11. Almost 80% of the patients completed the battery with all valid test results.

Patients (n)	Valid tests (n)	Percent
2	9	5.8%
1	10	2.9%
4	11	14.7%
27	12	79.4%
Total: 34	Max:12	

Table 11. Number of valid tests on the CBS battery per patient. Among all the 34 participants, 27 had all the 12 tests with valid results. Eight participants had 1, 2 or 3 invalid scores each.

The mean Z-scores and standard deviation (SD) of the participants of the study, matched by age, gender and educational level with the CBS normative database are shown in table 11.

The average of the cirrhotic population of this study showed a worse performance in 11 out of the 12 tests in comparison to the norms, represented by the negative mean Z-scores, and 6 of them were statistically significant ($p < 0.05$).

In each one of the tests, the percentage of patients who scored below the norms mean for each test is: 88% (Rotations), 79% (Paired Associates), 79% (Feature Match), 76% (Grammatical Reasoning), 70% (Polygons), 67% (Spatial Span and Spatial Planning), 64% (Double Trouble), 58% (Monkey Ladder and Digit Span) and 50% (Odd One Out and Token Search). The p values for each test are exposed in table 12.

Cognitive test	Valid/invalid results (n)	Mean Z score	SD	Z-scores below norms mean (n/%)	P value
Spatial Span	34/ 0	-.29	1.08	23 (67%)	.121
Paired Associates	34/ 0	-.75	.88	27 (79%)	< .001**
Spatial Planning	31/ 2	-.23	.69	23 (67%)	.066
Rotations	32/ 2	-.98	.87	30 (88%)	< .001**
Polygons	32/ 2	-.57	.96	24 (70%)	.002*
Feature match	33/ 1	-.52	.79	27 (79%)	< .001**
Odd One Out	33/ 1	.02	.76	17 (50%)	.836
Monkey Ladder	32/ 2	-.25	1.15	20 (58%)	.219
Grammatical reasoning	34/ 0	-.71	1.28	26 (76%)	.003*
Token search	34/ 0	-.06	1.26	17 (50%)	.762
Double Trouble	31/ 3	-.69	.99	22 (64%)	< .001**
Digit Span	34/ 0	-.32	1.12	20 (58%)	.100

Table 12. Patients' scores of CBS neuro-cognitive battery for each of 12 tests compared to normative database. The mean of the healthy population for all the tests is standardized at zero. Negative Z-scores show a poorer performance from the cirrhosis patients in this study in comparison to healthy individuals, matched by age, gender and educational level. *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed).

Figure 20 shows the participants individual performance on the complete CBS cognitive assessment, compared to the norms. The patients' mean was lower in all tests, and 6 of them were statistically significant ($p < 0.05$).

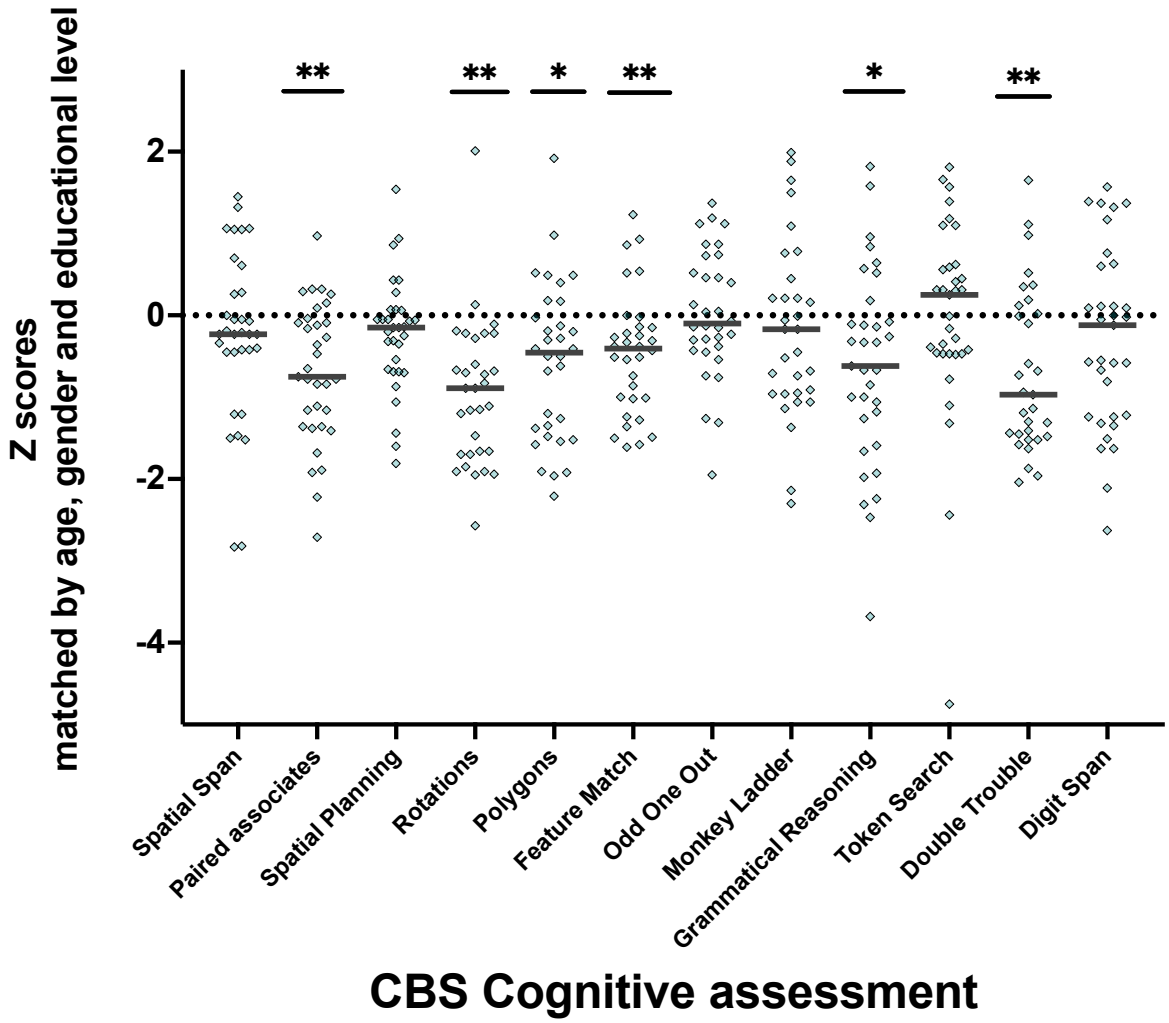


Figure 20. Participants' performance on the 12-test CBS cognitive assessment. All the means below zero show poorer performance in comparison to the normative database. *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed).

Previous studies have defined cognitive impairment if scores for each test were ≥ 1.5 SDs below age- and sex-matched controls from CBS normative database (Honarmand et al. 2019). Our study shows values ranging from 3,2% to 40,7% of participants with scores below 1.5 SDs (figure 21, table 13). All cognitive domains showed levels of impairment.

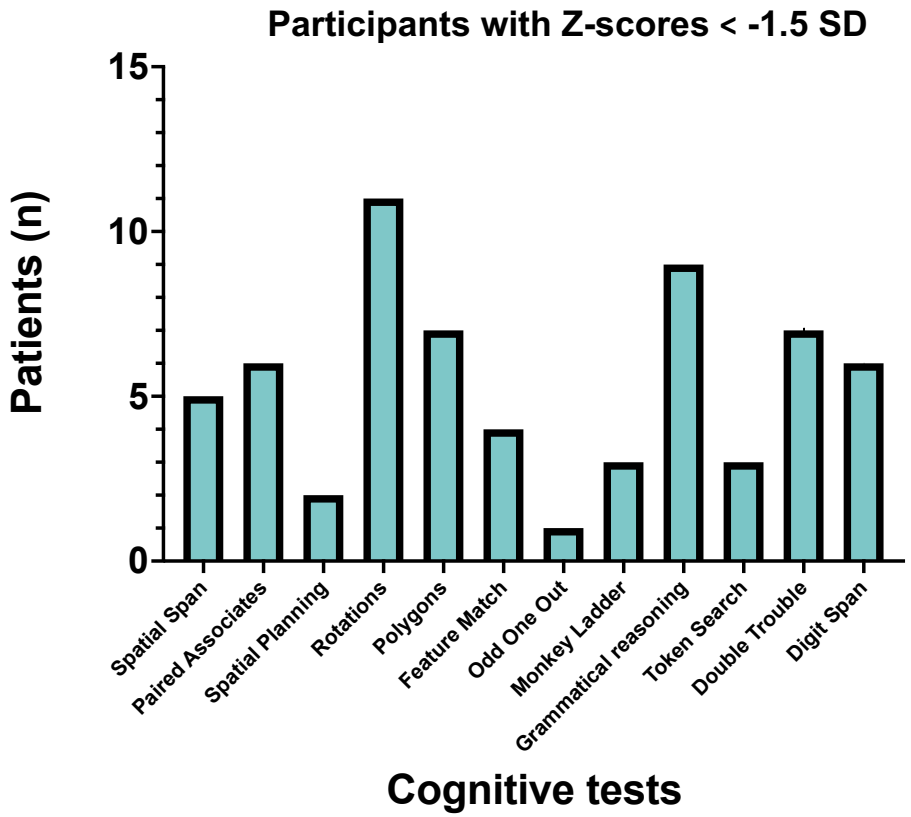


Figure 21. Participants who presented cognitive impairment at CBS battery. Representation of the number of participants who scored below -1.5 SD, defined as impaired for each one of the tests among the 34 participants of the study.

Percentage of participants with Z-scores < - 1.5 SD			
Spatial Span	16.1%	Odd One Out	3.2%
Paired Associates	22.2%	Monkey Ladder	10.7%
Spatial Planning	6.7%	Grammatical Reasoning	30%
Rotations	40.7%	Token search	9.4%
Polygons	25.9%	Double trouble	29.2%
Feature Match	14.8%	Digit Span	20.7%

Table 13. Percentage of participants with cognitive impairment in the CBS Battery.

The scores of the four tests with a higher percentage of cognitive impairment didn't show a correlation among them (table 14), which can suggest that the patients who failed one of these tests might not have failed the others.

	POL- impaired	ROT- impaired	DT- impaired
GR- impaired	.18 (ns)	.12 (ns)	.23 (ns)
POL- impaired		.15 (ns)	.10 (ns)
ROT- impaired			.17 (ns)

Table 14. Correlations between the scores considered as impaired for the 4 tests with a higher percentage of failure. The numbers in the table express Spearman's correlations. The p values are represented by ns= not significant. GR= Grammatical Reasoning, POL= Polygons, ROT= Rotation, DT= Double Trouble.

Figure 22 shows the distribution of the number of patients and the number of tests in which they were considered impaired, defined by a Z-score < - 1.5 SD in comparison to norms.

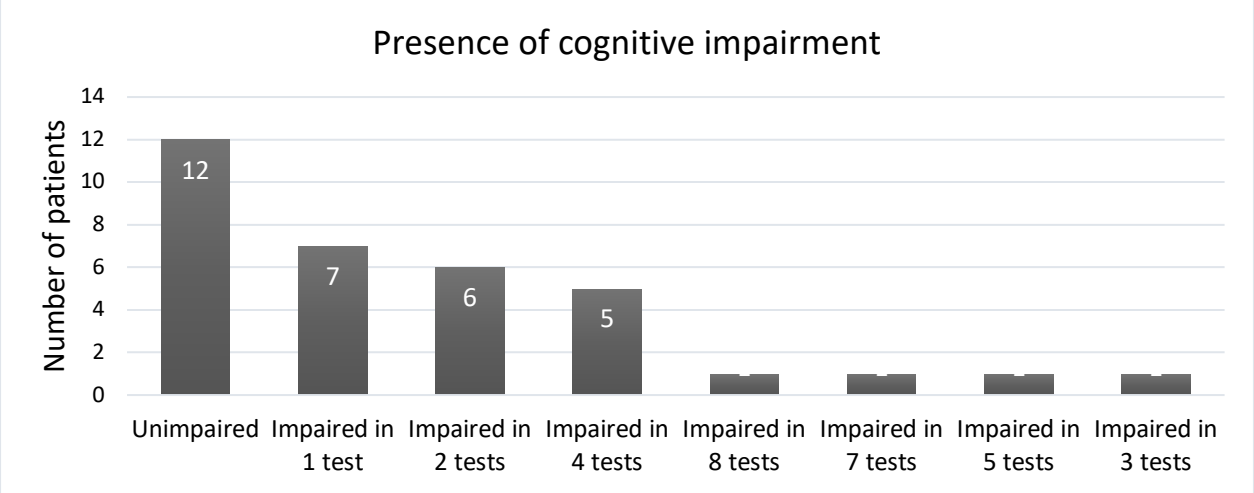


Figure 22. Prevalence of cognitive impairment in the study population using the CBS battery. Representation of the total number of participants (n=34) who scored < - 1.5 SD in comparison to the healthy population, what was defined as cognitive impairment in each test according to Honarmand et al. (2019).

Twelve patients (35%) didn't score bellow -1.5 SD in any of the 12 CBS tests. Twenty-two patients (65%) had Z-scores below - 1.5 SD, defining impairment. Seven patients were impaired in 1 test (in addition to 1 invalid test result in 1 patient), 6 patients were impaired in 2 tests (in addition to 1 invalid test result in 1 patient), 5 patients were impaired in 4 tests (1 patient with additional 3 invalid test results and 1 patient with an additional 1 invalid result), 1 patient was impaired in 8 tests (with 3 more invalid test results), 1 patient was impaired in 7 tests (in addition to 1 invalid test result), 1 patient was impaired in 5 tests (in addition to 2 invalid test results), and 1 patient was impaired in 3 tests.

If we considered the Z-score < - 1.0 SD below the norms as a definition of cognitive impairment for each test, 33 patients (97%) would be considered impaired in, respectively, 1 test (n=5), 2 tests (n=7), 3 tests (n=7), 4 tests (n=5), 5 tests (n=2), 6 tests (n=2), 7 tests (n=2), 8 tests (n=1), 9 tests (n=1). If we considered the Z-score < - 2.0 SD below the norms, 10 patients (29%) would be considered impaired in 1 to 5 tests.

4.2.1. Performance according to the cognitive domain

The scores according to the main cognitive domains (composite scores) were divided into reasoning skills, short-term memory and verbal processing, and represent the patient's average performance in that domain.

The participants of the study had lower performance in all domains in comparison to the norms ($p < 0.05$), defined by the presence of negative mean Z-scores, since the norms are represented by the mean= 0 and SD=1.

The total Z-score also reflected the impairment of the cirrhotic patients in comparison to the healthy population ($p < 0.001$) and represents their average performance across the entire battery of CBS tests (table 15, figure 23).

Cognitive domain	Mean Z score	SD	P value
Reasoning	-.16	.16	< .001**
Memory	-.13	.24	.003*
Verbal	-.15	.17	< .001**
Total score	-.44	.18	< .001**

Table 15. Performance of cirrhotic patients in the study divided by cognitive domain and total score in comparison to the norms. Negative Z-scores represent a worse performance of the participants in comparison to the norms (mean= 0, SD=1). *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed).

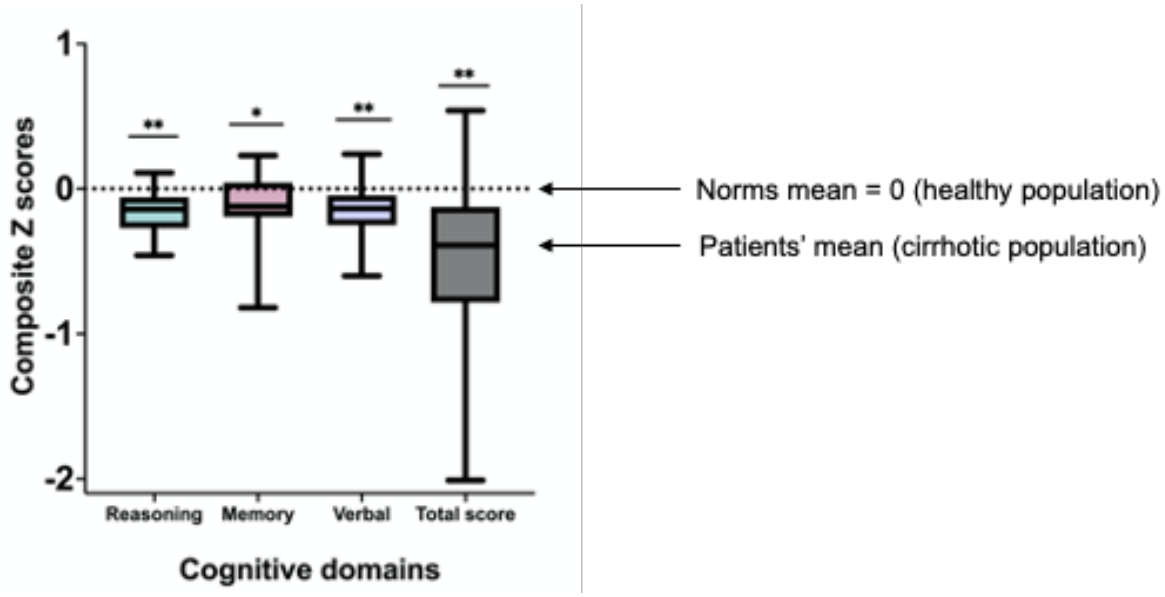


Figure 23. Patient performance on the CBS main cognitive domains compared to the norms. Results are matched by age, gender, and educational level. The bars represent the patients' mean for each cognitive domain, as well as the maximum and minimum values. *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed).

4.2.2. Correlation between tests, cognitive domains, and total Z-score

The 12 individual test scores correlated differently to each cognitive domain (table 16).

The correlations are as follows:

- Reasoning score:

Strong correlation: Spatial Planning, Polygons, Feature Match and Grammatical reasoning ($p < 0.001$),

Moderate correlation: Spatial Span, Monkey Ladder and Token search ($p < 0.001$),

Weak correlation: Paired Associates ($p < 0.05$).

- Memory score:

Strong correlation: Spatial Span, Paired Associates, Monkey Ladder, Token search ($p < 0.001$),

Moderate correlation: Spatial Planning and Grammatical reasoning ($p < 0.05$),

Weak correlation: Digit Span ($p < 0.05$).

- Verbal score:

Strong correlation: Grammatical Reasoning, Token Search and Digit Span ($p < 0.001$),

Moderate correlation: Spatial Span, Paired Associates, Spatial Planning and Polygons ($p < 0.05$),

Weak correlation: Feature Match, Monkey Ladder, Double Trouble ($p < 0.05$).

All cognitive domain scores correlated very strongly with the total score ($p < 0.001$) (figure 24).

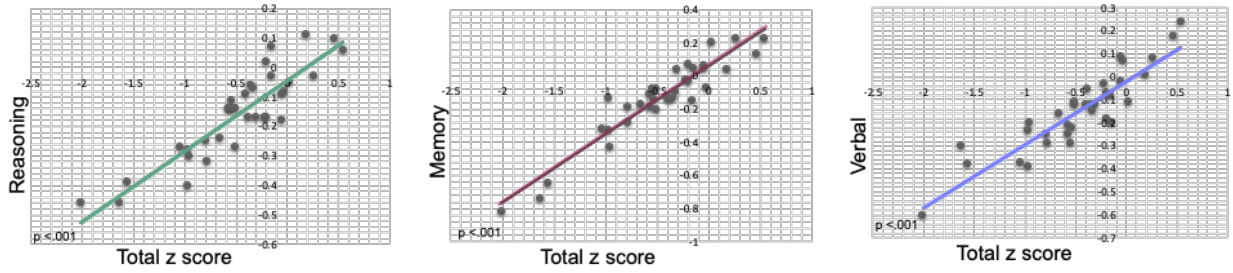


Figure 24. The linear correlation between the CBS cognitive domains and the total score. Participants' cognitive scores strongly correlated with the total score of the battery.

	Reasoning ρ	Memory ρ	Verbal ρ	Total ρ
Spatial Span	.52**	.70**	.43*	.58**
Paired Associates	.34*	.64**	.55**	.58**
Spatial Planning	.63**	.40*	.44**	.49**
Rotation	.29	.13	.20	.23
Polygons	.61**	.29	.47**	.52**
Feature Match	.64**	.32	.35*	.42*
Odd One Out	.24	-.01	-.09	.10
Monkey Ladder	.51**	.78**	.36*	.59**
Grammatical Reasoning	.61**	.49**	.73**	.68**
Token search	.45**	.72**	.68**	.73**
Double trouble	.19	.33	.38*	.29
Digit Span	.09	.39*	.63**	.48**
Reasoning				.84**
Memory				.89**
Verbal				.90**

Weak correlation | Moderate correlation | Strong correlation | Very strong correlation

Table 16. Spearman correlations for all 12 cognitive tests, the composite scores and the total score among the participants of the study. ρ= Spearman coefficient. Values between 0.80 and 1.0= very strong correlation; values between 0.60 and 0.79 = strong correlation; values between 0.40 and 0.59= moderate correlation; values between 0.2 and 0.39= weak correlation and values between 0 and 0.19= very weak correlation. Stars represent the statistical significance (p values). *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed).

4.2.3. Definition of abbreviated CBS scores

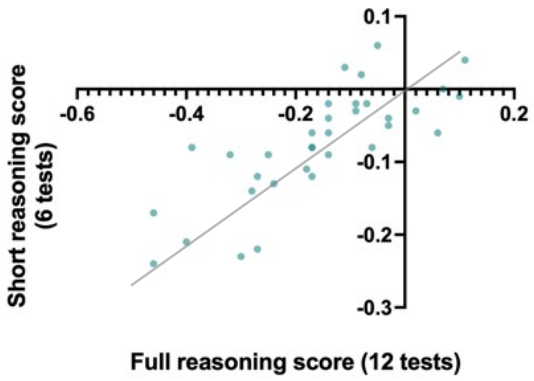
The abbreviated scores (short scores) were calculated using the 6 tests that most strongly correlated with the main cognitive domains (Hampshire et al. 2012).

The cirrhotic patients in the present study also showed to have worse performance in comparison to the norms in all short CBS scores, represented by negative mean Z-scores ($p < 0.001$) (table 17).

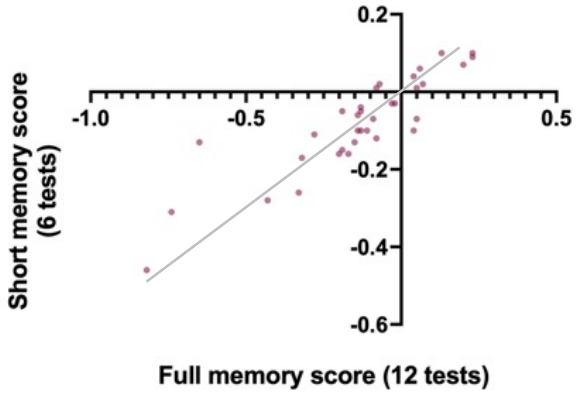
Cognitive domain	Mean Z-score	SD	P value
Short- Reasoning	-.07	.07	< .001**
Short- Memory	-.08	.12	< .001**
Short- Verbal	-.08	.13	< .001**
Short- Total score	-.23	.28	< .001**

Table 17. Abbreviated CBS scores: a short version of CBS battery. Representation of the cirrhotic population's mean Z-scores in comparison to the norms. Patients had worse performance than the healthy population in all short scores divided by cognitive domain and short total score.

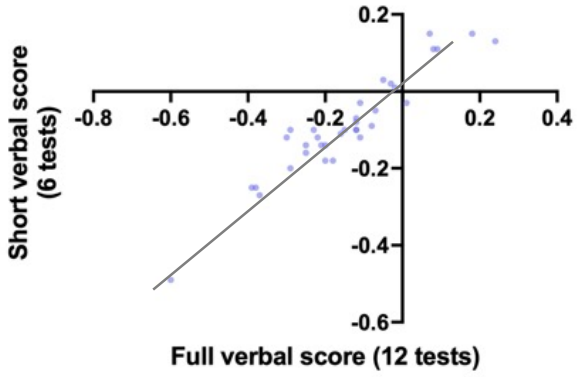
Figure 25 shows the correlation between the short and full scores divided by domain. All short scores (reasoning, memory, verbal and total) strongly correlated with the full scores ($p < 0.001$).



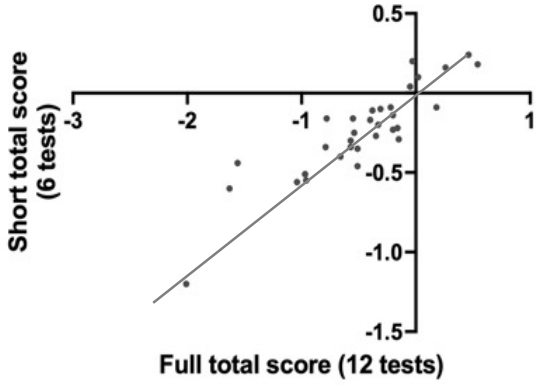
	Short reasoning score (6 tests)
Full reasoning score (12 tests)	.76**



	Short memory score (6 tests)
Full memory score (12 tests)	.87**



	Short verbal score (6 tests)
Full verbal score (12 tests)	.94**



	Short total score (6 tests)
Full total score (12 tests)	.89**

Figure 25. Correlations between short and full Z-scores from the cognitive assessment. The short Z-scores are calculated with 6 tests, while the full Z-score is the one previously calculated with the 12 tests. Numbers represent Pearson's coefficient (values greater than 0.7= strong correlation). Stars represent the statistical significance (p values). **Correlation is significant at the 0.01 level (two-tailed).

4.2.4. Correlation between cognitive domains and other variables of interest from baseline

The variables from baseline, including cirrhosis etiology, use of medication, cirrhosis complications, laboratory exams and Child-Pugh/MELD scores also correlated with the cognitive scores (table 18).

Bilirubin and hemoglobin moderately correlated with the memory scores ($p < 0.05$), and creatinine showed a moderate correlation with the verbal score ($p < 0.05$).

Bilirubin correlated weakly with reasoning, verbal, and total scores ($p < 0.05$).

	Reasoning	Memory	Verbal	Total score
Main etiologies of cirrhosis				
Alcohol	.10	.05	.05	.09
Viral hepatitis (B,C,D)	.27	.21	.64	.19
NASH	.16	.10	.18	.18
CHILD score	-.15	-.20	-.24	-.23
MELD-Na	-.25	-.33	-.16	-.24
Complications of cirrhosis				
GI bleed	-.27	-.27	-.22	-.27
Ascites	-.00	-.01	-.13	-.04
Varices	.12	-.10	.78	.01
HCC	.03	.01	-.15	-.05
Use of Psychotropic medication	.06	-.09	.11	.01
Laboratorial exams				
Hemoglobin	.28	.40*	.18	.27
Platelets	.05	.03	.07	-.00
Albumin	.18	.17	.16	.13
Total Bilirubin	-.36*	-.43*	-.36*	-.34*
Creatinine	.22	.16	.41*	.25
AST	-.01	.06	-.17	.00
ALT	.08	.09	-.01	.06
Sodium	-.07	-.14	-.02	-.16
INR	-.27	-.31	-.33	-.32

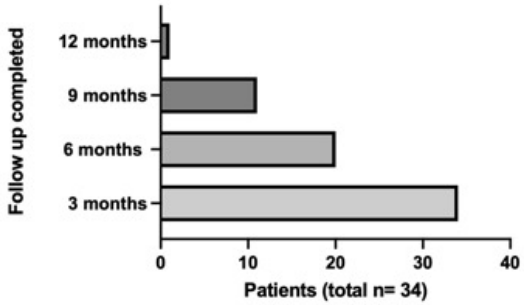
	Short Reasoning	Short Memory	Short Verbal	Short Total score
Laboratorial exams				
Hemoglobin		.44*		
Total Bilirubin		-.44**		-.41*
Creatinine			.42*	

Table 18. Correlations between laboratory exams from baseline, the cognitive domains and total score. Numbers represent the Pearson's coefficient (r): values greater than 0.7= strong correlation, values between 0.4 and 0.69= moderate correlation, less than 0.4= weak correlation. For the short scores, just the significant values are shown. Stars represent the statistical significance (p values). *Correlation is significant at the 0.05 level (two-tailed).

Similar results were found when compared to short scores. Other variables from baseline didn't show significant correlations with either the full or short cognitive scores.

4.2.5. Outcomes during follow-up

The end of 1-year follow-up for the final participants will be on October 31st, 2022. 3. On the date of January 31st, 2022, one patient finished the 12-month follow-up after the cognitive assessment at baseline. Eleven patients completed 9 months, 20 patients completed 6 months and all patients (n=34) completed the first 3 months. The follow-up status of the participants at this date is represented in figure 26.



Completed follow-up time on January 31, 2022	Patients (n/%)
3 months	34 (100%)
6 months	20 (58%)
9 months	11 (32%)
12 months	1 (2.9%)

Figure 26. Participants' follow-up status on January 31st, 2022.

According to the symptoms verified with the questionnaire applied by phone during the 3,6,9 and 12 months follow-up calls (annex 15), the patients reported changes in sleep pattern, mood, memory, and attention. Changes in sleep were classified as daytime sleepiness, insomnia, or non-specific changes. The memory loss was primarily due to short-term memory. Alterations in mood were stated as the presence of mood swings (figure 27).

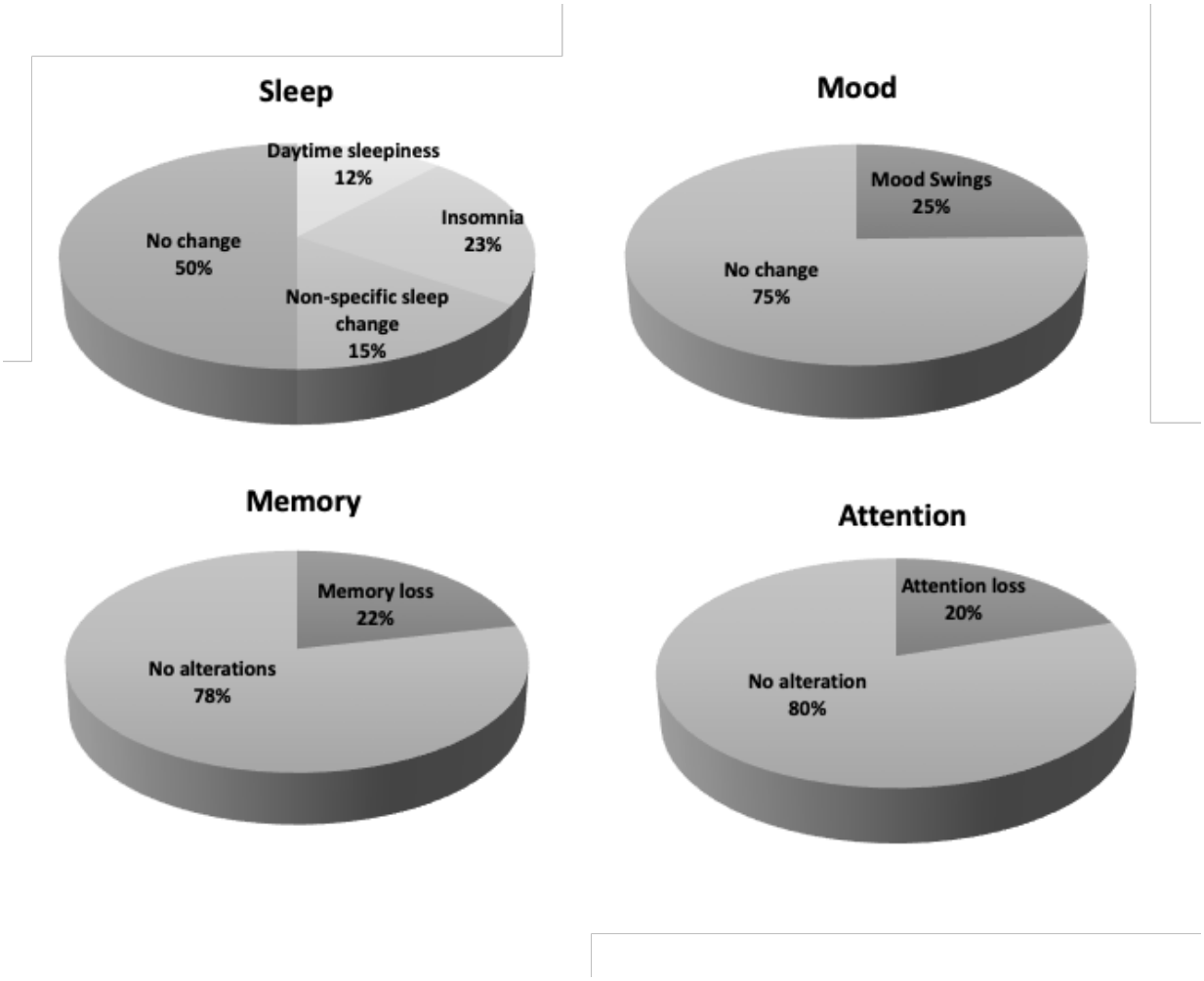


Figure 27. Symptoms reported by patients during follow-ups at 3,6,9 and 12 months.

According to the presence of complications of cirrhosis, other symptoms, and hospitalization-related causes, the results are displayed in figure 28. Constipation was a frequent symptom, reported 10 times, followed by fever (7 times). The presence of ascites was reported 4 times.

Hospitalizations related to HE happened 3 times. One patient was admitted because of abdominal pain, related to CLD, but no HE. Ten patients reported hospitalizations not related to liver disease.

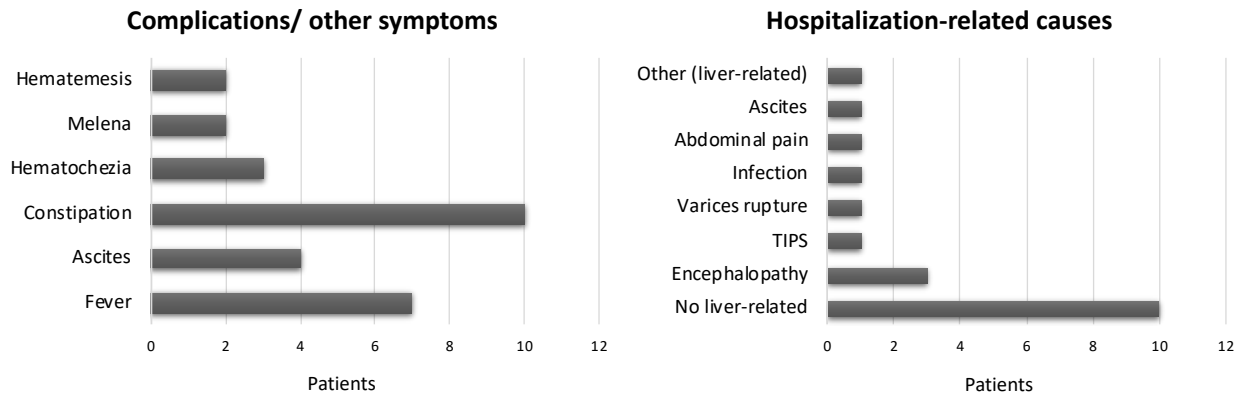


Figure 28. Complications, symptoms and hospitalization-related causes during participants' follow-up in the study.

4.3. Prevalence of overt hepatic encephalopathy

Until January 31st, 2022, 1 patient finished the study without OHE episodes. Three patients developed OHE, and all of them were classified as Child-Pugh A at baseline, with a mean MELD of 7. The main etiology of cirrhosis in OHE patients was NASH and the most prevalent complication at baseline was the presence of gastroesophageal varices.

During follow-up, all patients who developed OHE reported previous alterations in sleep, attention, and memory, and 2 patients reported uncommon alterations in mood. They also reported the presence of other possible precipitating factors before or during the hospitalization, like constipation, GI bleeding, TIPS placement and fever/infection (table 19).

Characteristics of the participants who developed hepatic encephalopathy during follow up n=3			
Baseline		**Follow-up (n)**	
Sex (male/female)	3/0	Attention alterations	3
Age	58.2 (54-61)	Sleep alterations	3
Cirrhosis etiology (n)		Memory alterations	3
Viral hepatitis	1	Mood alterations	2
NASH	2	Ascites	1
PBC	1	Constipation	2
Complications (n)		GI bleeding	2
Varices	2	Fever/ infection	2
HCC	1	TIPS placement	1
CHILD (A/B)	3/0		
MELD	7 (7-8)		

Table 19. Patients with OHE during follow-up. Characteristics present at baseline are exposed on the left, when characteristics developed during follow-up are exposed on the right of the table. Age and MELD are presented as mean, minimum and maximum values. NASH: Non-Alcoholic Steatohepatitis, PBC: Primary Biliary Cirrhosis, HCC: Hepatocellular carcinoma, MELD: Model for End-Stage of Liver Disease, GI: gastrointestinal, TIPS: Transjugular Intrahepatic Portosystemic Shunts.

Participants who developed OHE (n=3) had poorer performance in 6 out of the 12 tests at baseline (Rotations, Odd One Out, Monkey Ladder, Token Search, Double Trouble and Digit Span) and in the memory score in comparison to the participants' mean (n=34).

In comparison to the participant who didn't develop OHE until the end of the study (n=1), the OHE patients had poorer scores in 8 out of the 12 tests of the battery (Spatial Span, Paired Associates, Spatial Planning, Rotations, Monkey Ladder, Token Search, Double Trouble, and Digit Span), and in all cognitive domains and total scores.

The patient without OHE at the end of the study had higher scores than the participants' mean in 9 out of the 12 tests and in all cognitive domains/ total scores.

The scores from all participants (n=34), no OHE after 1 year (n=1) and OHE during follow-up (n=3) are exposed in table 20.

Z-scores divided by test			
	All participants	**No OHE**	**OHE**
Spatial Span	-0.29 (SD 1.08)	-0.07	-0.20
Paired Associates	-0.75 (SD .88)	0.97	-0.55
Spatial Planning	-0.23 (SD .69)	-0.07	-0.19
Rotations	-0.98 (SD .87)	-0.11	-1.00
Polygons	-0.57 (SD .96)	-1.20	0.03
Feature match	-0.52 (SD .79)	-0.54	0.00
Odd One Out	0.02 (SD .76)	-0.13	-0.10
Monkey Ladder	-0.25 (SD 1.15)	0.21	-0.87
Grammatical reasoning	-0.71 (SD 1.28)	-0.33	-0.24
Token search	-0.06 (SD 1.26)	0.31	-0.21
Double Trouble	-0.69 (SD .99)	0.35	-1.36
Digit Span	-0.32 (SD 1.12)	0.09	-0.52

Z-scores divided by cognitive domain and total score			
	All participants	**No OHE**	**OHE**
Reasoning	-0.16 (SD -.16)	-0.09	-0.12
Memory	-0.13 (SD -.13)	0.06	-0.15
Verbal	-0.15 (SD -.15)	-0.02	-0.12
Total score	-0.44 (SD -.44)	-0.05	-0.43

Table 20. Comparison between mean Z-scores in the group and patients with or without encephalopathy

The poorest performance of OHE patients was found in the CBS Stroop version (Double Trouble), where the OHE patients scored –1.36 versus –0.69 (participants' mean for this test), versus 0.35 (patient without OHE at the end of the study), followed by Rotations, where OHE patients scored –1.0 versus –0.98 (all participants), and -0.11 (patient without OHE).

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The time before the development of HE among the participants is shown in figure 29. The 3 patients developed HE within 3, 6 and 12 months of follow-up, respectively. Their total Z-scores didn't correlate significantly with the outcome.

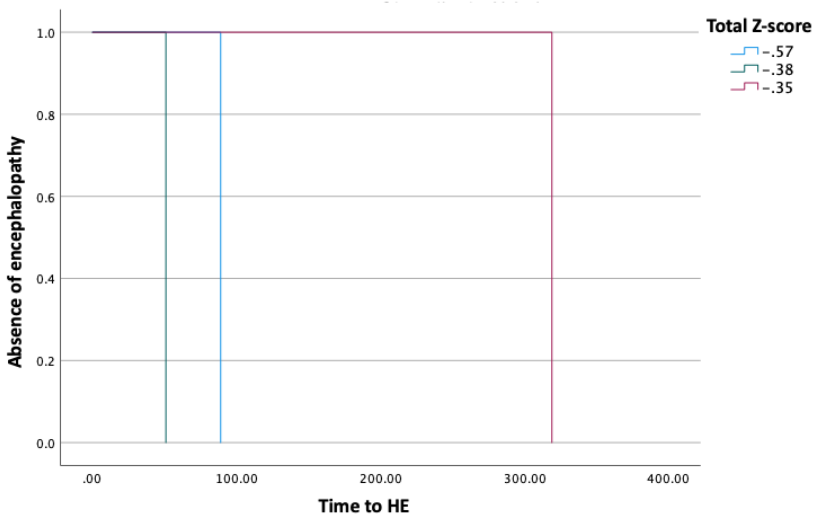

Figure 29. Time to development of encephalopathy among participants.

Linear regression models were performed to estimate the risk prediction of OHE but, up until the end of January 2022, no significant results were found.

We estimate that, at the end of the follow-up, we will be able to have the outcomes of the entire population of this study, which can allow us to perform the regression models properly.

Chapter 5. Discussion

This study performed an extensive evaluation of cognitive function in patients with cirrhosis using a web-based platform, designed for self-assessment at distance (at home), which demonstrated to be feasible in the context of COVID-19 restrictions and could be applicable in the clinical context by healthcare providers.

The battery of tests created by CBS allowed us to characterize the cognitive profile of patients with cirrhosis in great detail, observing which specific domains of cognition are impaired in this population and demonstrating how cognitive alterations can impact real-life activities, which has not been demonstrated in other studies.

The subclinical neurocognitive alterations found in cirrhosis are vast and don't follow the same patterns of progression towards the more advanced grades of HE. Investigating neurological dysfunction using numerous cognitive tests, which simultaneously evaluate multiple domains and functions, can provide us with important information about patterns of progression towards more advanced HE grades, which can ultimately better define risk prediction models.

5.1. Minimal/Covert Hepatic Encephalopathy

The majority of studies show a larger variability in the prevalence of minimal/covert HE, which ranges from 14% to 70% (Flud and Duarte-Rojo 2019). This variability reflects the multiple diagnostic tools available which evaluate different aspects and domains of the brain as well as the complexity and non-specificity of HE.

The subclinical neurological alterations, which are assessed by psychometric tests, can comprise of both cognitive and motor changes and progress to more advanced grades of HE, increasing the risk of negative outcomes. When compared with cirrhosis patients without MHE, those with subclinical HE are at higher risk of hospitalization and mortality (Patidar et al. 2014; Thomsen et al. 2016).

The presence of impairments related to CHE is associated with difficulties in maintaining professional activities, an increased risk of car accidents and a further deterioration

of socioeconomic status and quality of life. A better understanding of the cognitive/motor dysfunction and how these alterations translate to real-life situations can help patients and caregivers to adapt according to their options and prevent situations that can offer extra risks, such as driving and operating heavy equipment. Multiple studies have considered CHE assessment to determine these risks, since the patients usually are not aware if they have poor navigation skills related to CHE (Bajaj et al 2008, Shaw and Bajaj 2017, Formentin et al 2019).

Cognitive impairments in patients with cirrhosis are very frequent and are associated with the severity of the liver disease. Higher Child-Pugh scores are associated with a higher risk of suffering from CHE (Bale et al. 2018), which could suggest a need for screening, especially in these patients, who are also at higher risk to progress to OHE. These patients would benefit from a clear protocol of screening for cognitive impairment and possible treatment for high-risk patients. This variability reflects the multiple diagnostic tools available which evaluate different aspects and domains of the brain as well as the complexity and non-specificity of HE.

Some studies have shown that primary prophylaxis can be effective (Prasad et al. 2007; Sharma et al. 2012; Lunia et al. 2014), while others have shown a lack of effectiveness to prevent OHE in certain conditions, like the use of rifaximin as primary prophylaxis after decompensation by ascites, jaundice or variceal bleeding in patients with cirrhosis, for example (Sarwar et al. 2019). This suggests future studies are required to better define the moment as well as which patients' merit to be prophylactically treated (primary vs secondary). Consistent proof of improvement in outcomes following resolution of CHE using HE-targeted therapies is also a question that needs to be answered to justify primary prophylaxis.

It is also important to consider the course of CLD and the presence of pathogenic factors linked to HE, like ammonia, inflammation and oxidative stress levels, portal hypertension, risk of infection and others. These factors will be discussed in more detail in section 5.6. (overt hepatic encephalopathy). Effective treatments can minimize the impact of these factors, but not revert them completely, which certainly influences the resolution of HE.

The diagnosis between grade 0 (MHE) and grade 1 remains a challenge in clinical practice. For this reason, the recent guidelines (Vilstrup et al 2014) combined these entities and collectively defined them as CHE for clinical use. The identification of grade I is relevant, but the characteristics present at this stage are subjective and not specific. It is unlikely that clinicians

would apply consistently these criteria for CHE identification, especially because there's no wide consensus in the management of both grades 0 and I to date, or an approved treatment by the FDA (Food and Drug Administration).

Considering all medical records consulted for the present study, no register of minimal/ CHE testing was found.

5.2. Traditional psychometric testing

For many years, the need for early identification and treatment for CHE has been documented in multiple studies, since is leads to a higher risk of negative outcomes.

Traditional paper-pencil psychometric tests, such as PHES, have been used for a long time to define cognitive dysfunction in patients with cirrhosis. Multiple models (most of them computerized), that include neuropsychological and neurophysiological tests, were developed in the following years to facilitate access, and increase the applicability in the clinical setting: Scan test, Continuous Reaction Time test, Inhibitory Control Test, Critical Flicker Frequency, EEG and EncephalApp Stroop test.

Despite the large number of psychometric assessments already tested in the cirrhosis population to diagnose MHE, studies report that only 10% of clinicians send patients for a formal screening of CHE (Sharma et al. 2014). However, this rate can vary according to different healthcare institutions.

Different points of view between practicing clinicians and clinical researchers with an interest in HE may contribute to keeping the use of these tools largely underutilized. One reason is the lack of a formal definition of CHE and which tool(s) should be used to diagnose CHE. In addition, it is not clear when these tests should be conducted, if retesting is an option or, in case patients are diagnosed with CHE, what treatment should be provided (Flud and Duarte-Rojo 2019).

Beyond these factors, CHE has shown to be heterogeneous regarding the differences in clinical outcomes when comparing the presence of MHE and HE grade I, where hospitalizations and mortality rates are significantly increased in HE grade I than in MHE (Thomsen et al. 2016).

There is also a frequent disagreement on the definition of CHE defined by the diagnosis using different tests when applied to the same patient. This also reveals different prognostic values according to their results, which could suggest a combination of measurements to better define diagnostic and prognostic values (Montagnese et al. 2014). These facts contradict the tendency of developing faster and short cognitive assessments for CHE.

The traditional diagnostic tests for CHE don't assess always the same cognitive capacities and present different challenges to its performance and interpretation, which makes the diagnosis of CHE heterogeneous and not standardized, particularly because the tests require norms for each country to be widely used.

Paper and pencil-based tests, such as PHES, are the ones that assess the most cognitive abilities affected by cirrhosis, including motor speed, motor accuracy, concentration, (shift) attention, visual perception, visual-spatial orientation, visual construction, and memory. The tests are relatively easy to perform and have been validated/ translated into multiple languages/countries (Gabriel et al. 2021). However, it requires special staff to administer and interpret, which prevents it to be performed rapidly and conveniently in a clinic setting.

The Animal Naming Test assesses semantic fluency, verbal recall, and retrieval/self-monitoring of cognition. No equipment is required other than a stopwatch, and it is possible to perform at the bedside or clinical setting, but can be influenced by age and education levels (Campagna et al. 2017).

The computerized models, such as Scan test, Continuous Reaction time, Inhibitory Control test and Critical Flicker Frequency, emerged to simplify the diagnostic, allowing the tests to be performed in less time, but they assess fewer cognitive domains than PHES, and use different techniques to evaluate the impairments.

The Continuous Reaction Time test focuses on the evaluation of attention and inhibitory control through auditory discrimination, while the Critical Flicker Frequency, Inhibitory Control test, Scan test and EncephalApp Stroop test evaluate attention through visual discrimination. Some of these evaluate response inhibition as well while others don't (Luo et al.2019). These tests are not suitable for patients with hearing or visual impairments, according to the stimuli they provide to access the cognitive function. Advantage of the use of the Continuous Reaction Time test includes the limited influence of age, sex, or educational level

(Lauridsen et al. 2017). Critical Flicker frequency requires specialized equipment to be performed and can't be applied to persons with red-green color blindness (Kircheis et al. 2014). The Inhibitory Control Test requires highly functional patients and is influenced by previous exposure (Bajaj et al. 2008). The EncephalApp Stroop test is easy to administer using a tablet or smartphone, but can't be done in color-blind subjects and needs to be further validated in multiple countries to be widely used, as the other tests (Allampati et al. 2016).

The EEG evaluates the neural electrical activity, doesn't require adequate vision, hearing or motor strength and it's not influenced by learning effect, but requires neurological expertise to interpret and its evaluation is associated with inter and intra-observer variability, making the interpretation difficult and lacking objectivity (Amodio and Montagnese, 2015).

Besides the lack of homogeneity in the cognitive functions assessed and the techniques used by the multiple tests available, they also have different sensitivity to the diagnostic, and none of them are specific to CHE.

Multiple cognitive functions showed to be affected in CHE in this study and others and, unless we perform an extensive cognitive evaluation, it's not possible to define the areas more affected in each patient in the early stages. Most of the neuropsychometric tests also measure the patient's performance directly, which can be altered by multiple other factors, such as the use of certain medications, use of alcohol, lack of sleep, stress, performance anxiety and others. Exams like EEG can minimize the impact of some of these factors, since doesn't depend on the patients' participation to generate results, offering an objective evaluation of the mental status, but it is also not widely used.

All these facts exemplify the complexity of HE in its subclinical form, justifying why multiple diagnostic tools have been tested and others continue to be developed. But so far, none of them offered results able to justify well-established protocols of screening for all patients.

5.3. Cambridge Brain Sciences cognitive assessment

Over the past two decades, the battery of tests from CBS has been used to evaluate multiple domains of cognition (reasoning, memory, attention, and verbal ability), and have been applied to both patient and healthy populations (Owen et al. 1990, 1991, 1992, 1996, 2010; Bor

et al. 2003; Hampshire et al, 2012). Functional neuroimaging techniques were used to study the tests' neural correlation in healthy subjects (Owen et al, 1996) and in the context of neuropathology (Owen et al. 1998; Williams-Gray et al. 2007). The CBS tests have also been used for long-term monitoring of cognition (Duclos et al. 2020). In the past few years, the battery has been adapted so that patients can perform the tests online, unsupervised, allowing the execution of large-scale studies (Hampshire et al. 2012; Wild et al. 2018).

According to the available data, the CBS tests have been conducted over 10 million times, which produces one of the largest databases in this category (Duclos et al. 2020). CBS provides scientifically validated tests, which can detect minimal changes in cognition (Hampshire et al. 2012, Honarmand et al. 2019) and which are comparable to 2-3h traditional paper-pencil psychological tests.

The CBS test battery can measure the main elements assessed by traditional psychometric tests used to detect CHE, such as alterations in attention, alertness, response inhibition, executive functions, working memory, psychomotor speed, and visuospatial ability, allowing the production of a comparative panel about multiple domains affected in cirrhosis, simultaneously.

The low number of invalid results among our participants (3.1%, or 13 test results out of 408) demonstrates the platform was able to efficiently provide information so the patients could understand, interact, and perform the self-assessment remotely. This tool offers the advantage of not requiring professionals to apply or interpret the tests, assessing multiple cognitive domains and comparing them with results from thousands of healthy individuals. Furthermore, the CBS test results could be accessed in real-time, when the participant was performing the tests remotely, and data was stored securely on the web platform.

In the context of a pandemic, the patients were provided with the possibility of performing their evaluation at home, when they feel comfortable, having all the information they required to navigate through the screens, interacting with the tutorials and finishing their tests. The patients received instructions by email 1h before the time scheduled for the tests, pointing they should be alone in a room to perform the tests (annex 11), and they should not communicate with other person until they finish their evaluation. They also received a phone

call at the scheduled time to provide them the access to the platform and to confirm they were alone.

Even if some patients may find it difficult to adapt to this model of study due to a lack of computer skills, the patients who accepted to participate were able to finish the tests with satisfactory results according to their capacities, represented by over 96% of valid test's results. Just one patient didn't finish the complete battery of tests. These features facilitated us to conduct this study, finding a valuable option in the context of COVID-19 restrictions, and applying a high-quality, detailed cognitive assessment, which allowed us to identify the domains and functions which are more affected in the cirrhosis population of this study.

Beyond, we could also observe the diversity of impairments in cognitive sub-domains at an early stage and their pattern of presentation among the participants, who had heterogeneous subclinical alterations. This fact can justify the discrepancy between the diagnosis of CHE with other traditional tests for CHE, which is usually not explored in so much detail by other studies.

Limitations for the use of this method include the time to perform the complete battery, which can range from 35 to 45 minutes, the need for a computer or tablet with internet access and experience using a computer/tablet, and language limitations (tests are only available in English and French). Visual impairments and fatigue related to the duration of the testing can also be an issue. The CBS battery is also not validated in patients with cirrhosis for the assessment of CHE. Due to pandemic restrictions, we could not apply other traditional tests simultaneously to compare the results.

Accessibility to the CBS platform is easy and feasible in research and clinical settings, but fees apply. Access is via the CBS website, no downloads or software are required for the research team/health professional or patients.

Highly functional patients were required to participate in this study, which is performed entirely at distance. This fact may prevent the participation of many patients with more advanced stages of liver disease, who have also a higher risk of cirrhosis-related complications, including HE, which was observed in the present study. Multiple decompensated patients mentioned they were not feeling well to perform a cognitive assessment, preventing them from participating in the study.

Other studies used web-based psychometric test batteries to identify subtle cognitive impairments in patients with cirrhosis, like the Cogstate, evaluating psychomotor speed, attention, learning, and visual/verbal working memory with 9 tests (Cook et al. 2017), correlating with subtests of PHES. A battery of 5 “brain-training games”, administered on an iPad was also previously used in cirrhosis patients and has shown to be able to detect subtle impairments not detectable by subtests of PHES or even the ICT (Tartaglione et al. 2014).

The reliability of the CBS battery, which corresponds to the measure of the internal consistency of a group of items (or tests), was measured with the Cronbach's Alpha coefficient. The values obtained defined reliability.

5.4. Patient’s cognitive performance

5.4.1. General performance and comparison to the normative database

The results of the present study show that patients with cirrhosis tested poorly on the CBS tests when compared to healthy individuals in most of the psychometric tests.

The participants in this study had lower mean Z-scores when compared to the healthy individuals (normative database) in 11 out of the 12 tests performed. Six of them were found to be statistically significant, and are represented by Paired Associates, Rotations, Polygons, Feature Match, Grammatical Reasoning and Double Trouble. The main domains affected, according to the areas evaluated by these tests are, respectively: episodic memory, visuospatial processing, processing and interpreting visual information, attention, verbal ability and response inhibition. The features of each of these tests are described in detail in the next section (5.4.2).

The percentage of patients who had Z-scores below the norms’ mean for each of these tests ranged from 64% to 88%, which represents a high number of cirrhosis patients with worse cognitive performance than the healthy population matched by age, gender, and educational level. Considering the entire battery, the percentage of patients whose total score was below the norms’ mean ranged from 50% to 88%.

Most of the domains impaired in our study population are the ones altered in the traditional tests used in cirrhosis patients (Agrawal, Umapathy, and Dhiman 2015; Ridola, Cardinale, and Riggio 2018), which suggests the presence of CHE and, therefore, CBS could potentially be used to predict OHE development.

Over 79% of the participants (n=27) had 100% of valid test results, representing their understanding of the tasks proposed on the screen and proper interaction with the platform. Only 7 participants had 1 to 3 invalid results each, out of the 12 tests performed. These results show that patients were able to perform successfully the remote cognitive self-assessment online, reinforcing the applicability of this technique in research or clinical setting.

5.4.2. Activities impacted by test scores

The alterations found in each test can also be translated into daily activities, demonstrating which fields of cognition can be more affected in our participants. In this study, the lowest mean Z-scores of the group were found in Rotations, followed by Paired Associates, Grammatical Reasoning and Double Trouble. Daily activities related to the impairments in all subdomains assessed by the battery of tests are shown in table 21.

Cognitive Test	Domains	Examples of translation to daily activities
Spatial Span	spatial short-term memory	Driving, following and giving directions, searching for a lost item, learning a new dance move
Paired Associates	episodic memory	Linking up two items in memory, such as the type of object and its location, trying to find something, pairing new words with their meaning
Spatial Planning	planning	Fitting furniture into a car, then assembling it later, using reasoning and planning. A fundamental property of intelligent behaviour, planning can be easily affected by lifestyle, such as poor sleep
Rotations	visuospatial processing	Mentally holding a map of your environment in your mind, and rotating it lo align yourself in it

Polygons	process and interpret visual information	Picking out subtle differences between shapes, interpreting visuospatial information
Feature Match	attention	Identifying similarities and differences when comparing two things, to make choices
Odd One Out	deductive reasoning	Figuring out what it is true, based on a set of facts, using deductive reasoning
Monkey Ladder	visuospatial working memory	Holding information, manipulating, or updating it based on changing circumstances: viewing a situation, planning a sequence of moves, then execute them from memory
Grammatical Reasoning	verbal ability	Understanding verbal communication, what people are saying, even if they don't always communicate clearly. Understanding negative sentences: "I didn't know that he was not going to show up"
Token search	spatial working memory	Remembering clients who were already been visited from those who didn't, searching for a lost object accordingly to the circumstances
Double Trouble	response inhibition	Ability to block out background sounds/information when trying to focus on something else
Digit Span	short-term verbal memory	Remembering sequences of information, such as understanding longer phrases or remembering phone numbers when entering it at the phone

Table 21. Translation of CBS tests into daily activities. The test results can represent multiple subtle alterations, which can reflect different cognitive capacities necessary for common activities in the real context. www.cambridgebrainsciences.com.

The participants had different patterns of cognitive impairments, usually associated with the most affected domains in cirrhosis. Regardless, alterations were found in all domains evaluated. These results exemplify how the alterations can impact the patient's quality of life and decrease their capacity of performing usual tasks required in daily functioning, and how this could also impact their professional performance. It can also explain why the tests that measure specific domains may fail to provide the correct diagnosis of CHE, or prediction of OHE. As

previously demonstrated by other studies, PHES, the gold standard test for the diagnosis of CHE, is not sensitive enough to detect early neurological alterations in over 40% of patients, a relevant proportion (Gimenez-Garzo et al. 2017).

5.4.3. Definition of cognitive impairment

We defined cognitive impairment as Z-scores below –1.5 SD in comparison to the norms, a value used in previous studies (Honarmand et al. 2019). If we considered the Z-scores below - 1.0 SD as a definition of cognitive impairment, 33 patients (97%) would be considered impaired in 1 to 9 tests of the battery, while 10 patients (29%) would be considered impaired in 1 to 5 tests with the presence of Z-scores below - 2.0 SD.

Twelve patients (35%) scored above –1.5 SD in comparison to the norms in all 12 tests, being considered as non-impaired in the entire battery, while 22 patients (65%) had Z-scores below - 1.5 SD, defining impairment.

The presence of cognitive impairment ranged from 3.2% to 40% of the participants in each of the 12 tests/ domains evaluated.

Alteration in visuospatial processing represents the predominant cognitive dysfunction found (40% in Rotations and 25.9% in Polygons) and could be screened by PHES and RBANS.

Verbal ability corresponds to the second domain more affected in the population of the study (30% in Grammatical Reasoning), followed by response inhibition (29.2% in Double Trouble) and episodic memory (22.2% in Paired Associated).

There are multiple traditional tests capable to screen alterations in response inhibition, such as the Stroop EncephalApp, Inhibitory Control Test and Continuous Reaction Time, but the traditional tests usually don’t include the verbal ability evaluation, other than the Animal Naming Test, that measures semantic fluency, verbal recall and retrieval, that are not exactly the same features assessed by CBS, which measures the ability to quickly understand and draw valid conclusions about concepts expressed in words. The memory is assessed by multiple traditional tests, including PHES, RBANS, Scan test and Inhibitory Control Test.

Like other tests used for CHE diagnostic, none of these tests are specific, but they measure similar cognitive domains as the traditional tests.

In this study, 64% of the cirrhosis patients scored below the norms on the CBS Stroop test (Double Trouble), and around 30% of the patients were considered impaired in this test. Based on these results, we assume that patients with CHE in this study could reach values between 30% and 64%, if we consider only the Stroop test result, since we didn't compare CBS tests to traditional tests to define a cut-off for the CHE diagnostic.

If we consider the performance in the full battery, 88% of the patients scored below the norms in at least 1 test, and over 40% were considered impaired in at least 1 test, which could increase the percentage of patients with CHE.

Since a total of 22 patients (65%) had Z-scores below - 1.5 SD, we assume that CHE is present in all these patients.

Previous studies using the Stroop EncephalApp have found the presence of CHE in 37% to 54% of the patients, based on PHES and ICT, respectively (Allampatti et al. 2016). Consistent with previous studies, the participants scored significantly worse than healthy subjects in the Stroop task, which can indicate a lower psychomotor speed and impaired cognitive flexibility found in cirrhosis (Yang et al. 2018).

Since the traditional tests measure cognition subdomains similar to CBS, we expect that the CBS tests will be capable to predict the risk of progression to overt HE and correlate with some of the outcomes. However, there are domains shown to be altered in the CBS tests, that are not usually assessed by the traditional tests. This fact could justify the presence of patients undiagnosed for CHE by these tests, or a disagreement on the diagnostic by the traditional tests, which can also reflect in the prediction of the outcomes, as previously demonstrated by other studies (Montagnese et al. 2014; Allampati et al. 2016).

5.4.4. Performance divided by cognitive domains: reasoning, short-term memory and verbal ability

Each of the CBS tests evaluates a cognitive function as well as others simultaneously, since we use multiple brain areas and cognitive capacities to perform a task.

To calculate the composite scores, we considered the contribution of all 12 tests to each specific domain (factor loading), as previously determined. The study by Hampshire et al

(2012) mapped the brain networks used to perform the tasks using MRI. The activation level of each voxel in the brain cortex was calculated for each one of the tests relative to a resting baseline state. The factor loading of each test was calculated and used previously (Wild et al. 2018) and reflects the contribution (component weight) of each test to each domain (reasoning, short-term memory, and verbal ability).

The results show that, when divided by cognitive domains (reasoning, short-term memory, and verbal ability), the participants' performance was also below the norms. Their Z-scores values ranged from -0.13 to -0.16, which doesn't reflect a significant difference in impairment between the main domains evaluated. Reasoning was the domain with the lowest score among the participants.

All domains' scores correlated strongly with the total score, which reflects the overall performance of all battery. The individual tests also correlated with the domains differently, reflecting their association with different cognitive functions assessed.

In this study, Feature Match is the test that most strongly correlated with reasoning, followed by Spatial Planning, Polygons and Grammatical reasoning. Monkey Ladder strongly correlated with memory, followed by Token Search, Spatial Span and Paired Associates. Grammatical reasoning also strongly correlated with the verbal score, followed by Token Search and Digit Span. Some of the tests correlated strongly with more than one domain, reflecting their domains assessed during the task. These correlations don't necessarily reflect the factor loading of each test for its respective domain.

Previous studies reported that patients with CHE had worse performance than patients without CHE in motor performance and cognitive functioning, including learning, memory, and verbal fluency (San Martin-Valenzuela et al. 2020). Memory scores were previously found to be lower in patients without CHE in comparison to healthy subjects, and even lower in patients with CHE in comparison to patients without CHE (Bahceci et al. 2005), which can also reflect the results of the present study.

5.4.5. Definition of abbreviated battery scores

Previous studies reported patients' fatigue when performing the full battery of tests (Honarmand et al. 2019). However, in this study, the patients were critical illness survivors in the Intensive Care Unit, which doesn't reflect the same conditions as our participants. The use of an abbreviated battery, in our context, could be useful to offer faster options to calculate the risk of OHE.

The abbreviated scores, containing just the 6 tests that most strongly reflect their respective cognitive domain, showed to strongly correlate with the full scores. This result is particularly interesting when there's a need to reduce the time to perform the self-assessment, considering less functional patients.

5.5. Relation between test scores and variables from the baseline

Considering the relation between the test performance and laboratory exams from baseline, we found a moderate correlation between higher levels of bilirubin and lower performance in memory. The same correlation was found between low levels of hemoglobin and memory. High levels of creatinine, reflecting the decrease in renal function, had a moderate correlation with the verbal score.

These results suggest that the progression of liver disease, reflected by increases in total bilirubin levels and the presence of anemia can cause an impact on the field of short-term memory in cirrhosis patients, and a deterioration in kidney function would have an impact on verbal ability. Similar correlations were found for the short scores. Other studies have demonstrated the association between these biomarkers with cognitive impairment and/or risk of developing HE in cirrhosis. Albumin was found to be associated with cognitive impairment in the early stages of HE, and levels below 3.05 g/dL were effective to identify cognitive dysfunction in patients aged ≤65 years without portosystemic shunt (Kaji et al. 2021). The authors recommended the albumin as a screening test before applying the traditional psychometric tests.

Anemia, defined by low hemoglobin levels, was associated with cognitive function and considered a predictor of OHE (Kalaitzakis et al. 2013). Anemia was defined as blood

hemoglobin <117 g/L in women and <134 g/L in men. In the same study, HE was also independently related to renal impairment and systemic inflammation.

Albumin-bilirubin (ALBI) scores, associated with the level of liver fibrosis, were capable to identify patients with cirrhosis patients at risk of the first decompensation, which includes the development of HE, gastrointestinal bleeding, and ascites (Guha et al. 2019). The ALBI score was also found to be a predictive test for post-TIPS encephalopathy in a cohort of 82 cirrhotic patients within 1 year after TIPS placement (Lin et al. 2021).

No other significant correlations were found in this study between the test scores and cirrhosis etiology, use of psychotropic medication (antidepressants) or any of the complications of CLD.

Child-Pugh and MELD scores, which reflect the severity of liver disease, didn't correlate with the cognitive performance in this study, as previously found (Agrawal et al. 2020). Since we didn't perform a formal evaluation to access HE grade I at baseline in our participants, all the patients were classified as "no encephalopathy" during the calculation of Child-Pugh score.

Risk related to medication was previously found with benzodiazepines, opiates, proton pump inhibitors, GABAergics and others (Tapper et al. 2019).

Chronic antidepressants, however, were not associated with cognitive impairment in prior studies (Bajaj, Thacker, et al. 2012).

Other studies have also shown an increased deterioration of cognitive status in patients with alcoholic liver disease in comparison to other etiologies. This association can be explained by the fact that alcohol is also a neurotoxin, and patients not always can be abstinent after the diagnosis of cirrhosis, which contributes to further impairments in cognition and liver disease severity over years. In our study, we didn't find significant correlations between the variables from baseline, like cirrhosis etiology, presence of previous decompensation events, use of medication, laboratory exams and others with the composite scores (reasoning, memory, and verbal ability).

As a complication that results from multiple pathological pathways, HE may count with multiple risk-related associations, as demonstrated previously (Taper 2019). However, the numerous factors related to the pathogenesis of don't always cause the same consequences in all patients, which can make screening and prevention a challenge for clinicians.

5.6. Overt Hepatic Encephalopathy

OHE manifests with a dynamic spectrum of severity. It is, certainly, the most complex of all complications of cirrhosis, presenting with unpredictable neurocognitive alterations, progressive disability, and a high risk of other negative outcomes.

As previously pointed out, the early identification of a patient's risk for OHE may allow certain actions to prevent further deterioration, such as closer monitoring, lifestyle adaptation, earlier treatment, and prevention of well-known complications, such as falls and motor vehicle accidents (Bajaj, Pinkerton, et al. 2012).

The end of the study, when all participants will have finished their follow-up, will be on October 30, 2022. Until January 31, 2022, 1 patient finished the 12-month follow-up without HE, while 3 patients (8.8%) developed OHE within 3, 6 and 12 months of follow-up, respectively.

The patient without OHE at the end of the study didn't fail any test and had higher scores than the participants' mean in 9 out of the 12 tests and in all cognitive domains/ total scores. The patient also related subtle alterations in short-term memory during the follow-up and had a liver-related hospitalization, but no HE.

All the 3 patients with OHE related alterations in sleep, memory, and attention, and 2 of them related mood alterations, before the development of the OHE episode. They scored below the mean of all patients in 6 to 7 out of the 12 tests of the battery and had lower scores in the CBS version of the Stroop test (Double Trouble). In comparison to the patient who didn't develop HE, the patients with OHE had lower Z-scores in 8 out of the 12 tests of the CBS battery and in all cognitive domains (reasoning, memory, verbal) and total scores.

The lowest Z-scores of OHE patients were found in the CBS Stroop version (Double Trouble), followed by Rotations, Monkey Ladder, Paired Associates, Digit Span and Token Search, where the OHE patients scored significantly worse than the patient without HE at the end of the study.

The risk prediction models didn't provide us with significant results at this point. At the end of follow-up for all participants, at the end of October 2022, we will be able to analyze the results related to HE risk prediction for all 34 patients. We aim to identify if the test scores, or even one of the tests, can better predict an OHE episode in 1 year, and perform the regression

models to identify other clinical factors or biomarkers available in this study, related to the outcome.

It was previously mentioned that OHE can be found in around 30 to 40% of patients with cirrhosis, with a 1-year risk of development of 20% (Bajaj 2010), and 10% to 50% of patients after TIPS (Poordad 2007). We believe the numbers in our study didn't reach similar values since the follow-up is still ongoing.

It has been previously mentioned that clinically apparent HE is a result of the combination of multiple factors (Tapper 2019):

- The severity of liver disease: quantified by CHILD and MELD scores, it represents the main leading factor of HE, since is related to the progression of the pathogenic processes that directly increase the risk of multiple complications,

- The presence of CHE: a risk factor for the progression through overt grades of HE, measured by the neuro-psychometric/ neurophysiological tests,

- The level of portal hypertension, related to the presence of varices/ bleeding, thrombocytopenia (Lens et al. 2015), that can be measured by portal manometry,

- Systemic inflammation status, usually linked to the gut bacteria in cirrhosis and to the development of cognitive impairments (Montoliu et al. 2009; Shawcross et al. 2011), measured by the gastrointestinal microbiome and inflammatory cytokines,

- Sarcopenia, assessed by imaging techniques, related to liver disease progression and the role of skeletal muscles in the ammonia metabolism,

- Use of psychotropic medication, such as benzodiazepines, or other classes that can interfere with the metabolism of the HE-risk pathway, like proton-pump inhibitors (which can alter the gut microbiome), metformin (modulating enteric glutaminase activity), and opioids (altering gut motility) (Acharya et al. 2017; Bajaj et al. 2018).

It is not possible to dissociate the risk of HE with the factors mentioned above, since all of them contribute to its development. Involving multiple pathological pathways and manifestations in comparison with other decompensation events of cirrhosis, HE diagnosis and prediction models could also reflect this complexity, considering the presence of these events.

5.7. Future predictive models

Hepatic encephalopathy is a complication that can reflect on clinical, laboratory, electrophysiological, and imaging study alterations. Many different variables can be considered for risk prediction simultaneously, including the cognitive assessment. As we observed in our study, where we explored multiple domains of cognition and their alterations in a population of cirrhosis, different areas are affected among the patients, reflecting different impairments in cognitive function and, probably, this can also reflect on the OHE risk-prediction.

The traditional tests measure different domains, and patients have different patterns of cognitive domains impaired. If a certain test is not able to detect impairment in a certain domain, the diagnosis of CHE would be missing.

If we can establish a clear correlation between some factors (including etiology, laboratory exams, presence of decompensation events, disease severity) and a specific type of early cognitive impairment, tests can be designed or chosen accordingly. Requiring a more complete evaluation of cognitive function could also offer a solution to properly screen all types of alterations.

Future studies should consider the diversity impairments in cognition to screen the patients for CHE as well as their role in predicting the risk of developing OHE. The study of cognitive monitoring should also be considered for future studies, once cognition responds dynamically according to clinical and biochemical changes involving the pathways to HE.

Chapter 6. Study challenges and limitations

As an adaptation to the restrictions imposed by the COVID-19 pandemic, this study had some important limitations. The first challenge was related to the recruitment, which was done entirely at a distance. This study was difficult for patients to accept and demanded their active participation using resources such as email, phone calls, online calendars, and documents.

The retrospective collection of biochemical data from previous laboratory results (not older than 6 months before enrollment), may also not represent the biochemical status of the patient at the time of the CBS test (baseline for all participants). However, it was impossible to draw blood and run biochemical analysis virtually. The patients were also not pre-evaluated for red- green color blindness or dementia. They were questioned about possible visual, cognitive or motor impairments that could prevent them to participate in the study during phone interview, as the information was also checked in medical records previously. However, if the patient was undiagnosed concerning these conditions, they could not be excluded.

Concerning the tests execution, information by email and by phone was provided, respectively, 1h before and at the scheduled time for performing the tests, to ensure the patient was alone in a silent room to complete the evaluation by themselves, without interaction. The progression of tests execution could be followed in the CBS platform in real time, but we can't be sure all the patients performed the tests alone.

The follow-up by phone is based on the patient's description of their health state. They may lack information related to the complications, causes of hospitalization, medication in use (especially in advanced age), presence of cognitive impairment or more advanced stages of cirrhosis. Sometimes, the information provided by the patient during follow-up is not the same found in his medical records, for this reason, all information provided by the patient was confirmed on their medical records, but this remains as a possible origin of bias.

In addition, many patients were not residents of Montreal and subsequent hospitalizations may occur outside the CHUM, therefore it was important to ask whether any patients were hospitalized outside the CHUM during the follow-up call.

The participation of hepatologists in the patients' referral increased their acceptance of this study, which may indicate a benefit of doctors' participation in clinical projects during recruitment.

The presence of complications of cirrhosis and severity was a factor which impacted the patients' acceptance to participate in this study, as they mentioned multiple times when contacted by phone. The online self-assessment was better accepted by patients with better health conditions (less disease severity), reflecting, probably, a better cognitive function as well. This could lead to a bias in the patients that decided to participate.

Due to the pandemic restrictions, time limitation was a considerable factor, and for this reason, the follow-up is still ongoing. We experienced a delay of around 9 months due to the restructuring of the project, changes in protocol, and later difficulties related to recruitment. This study is underpowered and therefore a higher number of patients included in the study would allow us to statistically run all the analyzes and possibly find more significant results.

Ideally, the CBS battery could have been applied simultaneously with other traditional tests for the diagnosis of CHE to compare results and correlate with laboratory markers and clinical outcomes in a larger population, and pre-evaluation tests to exclude dementia and red-green color blindness could have been done. If the results were satisfactory, a remote cognitive assessment could certainly be a very good option for many patients and their healthcare professionals.

Chapter 7. Conclusion

Following the tendency of using innovative technologies, this study used a web-based tool to perform a remote self-assessment in patients with cirrhosis, which was feasible and efficient in the context of COVID-19 restrictions, and which could be used in the future for home monitoring of cognitive function.

This battery of tests created by the CBS allowed us to evaluate in great detail different cognitive domains which have not been explored with other neuropsychometric tests used in HE. We could also define the main fields of cognition that are usually affected by cirrhosis, which have important impacts on patients' quality of life, daily activities, and can predict the onset of OHE.

Our results also show in part why many traditional tests show a discrepancy in their diagnosis and prediction of OHE in cirrhosis since the patients present with different parameters of cognitive impairments which varies depending on the test used. Furthermore, the newer tests are becoming more and more “simpler” in order to reduce testing time and the required expertise to analyze results. This fact can lead to a decreased sensitivity and specificity.

According to previous studies and our results, cirrhosis patients with early cognitive impairments showed to have deficits in different fields of cognition, not following a pattern of cognitive domains affected, which have shown to be heterogeneous among the participants.

The cognitive profile is proven to be very important for risk prediction of OHE. However, it’s one of the multiple measurements that reflect the severity of the liver disease and its multiple pathological pathways that lead to an OHE episode.

After many years of trying to find better screening tests for CHE, it’s possible to observe that there is still a lack of consensus on what test(s) to use, at what point in disease’s development patients should be tested, if tests are supposed to be repeated over time, what treatment measures to adopt in case of a positive test, what elements should be present to justify primary prophylaxis and others.

The future challenge is to evaluate the cognitive function as a continuum with clinically relevant outcomes to predict this decompensation event and justify primary prophylaxis in patients at high risk, using effective and well-tolerated treatments.

Ideal predictive models could indicate regular examination protocols, making screening a routine for the follow-up of patients with cirrhosis.

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Chapter 9. Annexes

Some of the official documents are available in French. All documents sent to the patients were available both in French and English and were sent according to their preference.

Annex 1. Ethics Committee approval

Comité d'éthique de la recherche du CHUM
Pavillon R, 900 rue St-Denis, 3e étage
Montréal (Québec) H2X 0A9

Le 1er mai 2019

Docteur Christopher Rose
Axe de recherche : cardio-métabolique

Objet :	**19.029 – Approbation FINALE (Évaluation déléguée)**
	Tests psychométriques chez des patients cirrhotiques

Docteur,

Nous accusons réception des précisions et corrections demandées ainsi que des documents suivants en vue de l'approbation finale du projet mentionné en rubrique:

formulaire d'information et de consentement français modifié – principal - version 2 du 1er mai 2019
formulaire 20 complété
protocole propre version 2 du 30 avril 2019
protocole suivi des corrections version 2 du 30 avril 2019

Le tout étant jugé satisfaisant, il nous fait plaisir de vous informer que la présente constitue l'approbation finale de votre projet de recherche, **valide pour un an à compter du 1er mai 2019.**

Vous devrez compléter le formulaire de renouvellement que nous vous ferons parvenir annuellement. De même, vous devrez soumettre pour approbation préalable, toute demande de modification ou document de suivi requis par le comité d'éthique conformément à ses Statuts et Règlements et ce via Nagano.

Vous retrouverez dans Nagano section "Fichiers – FIC – version approuvée CÉR CHUM (pdf)" une copie du formulaire de consentement portant l'estampille d'approbation du comité. Seule cette version finale devra être utilisée pour signature par les participants à la recherche.

Veuillez noter que le projet de recherche ne pourra débuter avant que vous n'ayez reçu la lettre de la personne mandatée pour autoriser cette recherche dans les murs de l'établissement. De même, lorsque cela s'applique à votre situation, le projet ne peut débuter tant que le contrat n'est pas finalisé et dûment signé.

Le comité d'éthique du CHUM est désigné par le gouvernement du Québec (MSSS) et adhère aux règles de constitution et de fonctionnement de l'Énoncé de Politique des trois Conseils (ÉPTC 2) et des Bonnes pratiques cliniques de la CIH.

Pour toute question relative à cette correspondance, veuillez communiquer avec la personne soussignée via NAGANO, ou avec le secrétariat du comité par téléphone ou courriel: ethique.recherche.chum@ssss.gouv.qc.ca – 514 890-8000, poste 14485, ou consulter le fichier «Questions-réponses» au bas de la page d'accueil Nagano**.**

Vous souhaitant la meilleure des chances dans la poursuite de vos travaux, nous vous prions d'accepter, Docteur, nos salutations distinguées.

M. Yves Poirier
Vice-président
Comité d'éthique de la recherche du CHUM

Annex 2. Information and Consent Form

CHUM

APPROUVÉ – CÉR CHUM
DATE: 30 juillet 2021
INITIALES: YP

FORMULAIRE D'INFORMATION ET DE CONSENTEMENT

Titre du projet:	Tests psychométriques chez des patients cirrhotiques
Chercheur responsable:	Christopher F. Rose, Ph.D., professeur titulaire, Département de médecine, Université de Montréal. Chercheur régulier, CRCHUM
Financement:	Fonds de recherche du chercheur responsable
No de projet au CHUM:	19.029

PRÉAMBULE

Nous sollicitons votre participation à un projet de recherche parce que vous souffrez d'une maladie chronique du foie. Cependant, avant d'accepter de participer à ce projet et de signer ce formulaire d'information et de consentement, veuillez prendre le temps de lire, de comprendre et de considérer attentivement les renseignements qui suivent.

Ce formulaire peut contenir des mots que vous ne comprenez pas. Nous vous invitons à poser toutes les questions que vous jugerez utiles au chercheur responsable du projet ou aux autres membres du personnel affecté au projet de recherche et à leur demander de vous expliquer tout mot ou renseignement qui n'est pas clair.

NATURE ET OBJECTIFS DU PROJET

Ce projet de recherche consiste à étudier le lien entre le foie et le cerveau par le biais de tests psychométriques. Un test psychométrique est une évaluation objective d'un trait psychologique comme par exemple la mémoire, la concentration, l'aptitude, etc. De nouveaux tests en ligne ont été élaborés. Ils sont abordables, faciles d'utilisation et ne prennent que 35 à 45 minutes comparativement aux tests psychométriques traditionnels sur papier et stylo d'une durée de 2 à 3 heures.

Le but de cette étude est de valider les nouveaux tests psychométriques disponibles en ligne, chez les patients cirrhotiques.

NOMBRE DE PARTICIPANT(E)S ET DURÉE DE LA PARTICIPATION

Dans le cadre de cette étude, nous souhaitons recruter 190 participants au CHUM.

La durée totale prévue pour l'étude est de 18 mois. La durée de votre participation consistera en une seule séance de 35 à 45 minutes.

NATURE DE LA PARTICIPATION DEMANDÉE

Si vous acceptez de participer à cette étude et après avoir signé le présent formulaire, nous vous demanderons de compléter une évaluation en ligne d'environ 35 à 45 minutes. Il s'agit de tests sous forme de jeux vidéo interactifs en ligne. Vous serez installé chez vous dans une pièce fermée. Cet environnement confidentiel et tranquille permettra de vous concentrer afin de répondre aux questions et de faire les tests.

DÉROULEMENT DU PROJET/PROCÉDURES

Cette étude comportera 3 phases:

1) La période de détermination de l'éligibilité (5 minutes)

Une personne de notre équipe a communiqué avec vous et a vérifié votre intention de participer à ce projet de recherche. Vous recevez donc le présent formulaire car vous répondez aux critères d'admissibilité suivants:

1. Avoir un diagnostic établi de cirrhose (biopsie ou fibroscan)
2. Être âgé de 18 ans et plus
3. Être capable de parler français ou anglais
4. Être capable de donner un consentement informé
5. Accès à internet avec familiarité suffisante pour l'utiliser

Vous ne pourrez pas être éligible si vous avez un ou l'autre de ces points :

1. Avoir l'encéphalopathie hépatique manifeste au moment du test ou un historique d'épisodes d'encéphalopathie hépatique
2. Avoir subi une greffe du foie
3. Avoir toute maladie neurologique
4. Être actuellement sous thérapie avec des drogues psychoactives
5. Avoir fait usage de drogues illicites dans la dernière année
6. Avoir un abus d'alcool passé ou présent selon la définition psychiatrique : Une histoire de consommation d'alcool qui résulte en une ou plusieurs des situations suivantes dans les 12 derniers mois:
    a. Incapacité majeure à remplir ses responsabilités au travail, école ou à la maison
    b. Boire dans des situations physiquement dangereuses, comme en conduisant une auto ou opérant de la machinerie lourde
    c. Avoir des problèmes légaux reliés à l'alcool, comme avoir été arrêté au volant sous l'influence de l'alcool ou pour avoir blessé quelqu'un en état d'ivresse
    d. Continuer à boire malgré les problèmes relationnels causés ou empirés par la consommation excessive d'alcool.
7. Avoir une histoire de trauma crânien résultant en une perte de conscience de plus de 30 minutes

2) La séance d'évaluation aux tests psychométriques (environ 35 à 45 minutes)

Voici les étapes à suivre pour l'évaluation psychométrique:

- Après votre consentement, notre équipe de recherche vous enverra un lien par e-mail qui vous donne accès à l'évaluation.
- Avant de commencer, vous serez invité à consulter les instructions et les détails concernant les étapes à suivre pour effectuer votre évaluation, ainsi que la durée de l'évaluation.
- Après avoir lu les instructions, vous passerez au didacticiel interactif. Vous pouvez répéter le didacticiel plusieurs fois pour vous assurer que vous êtes à l'aise avec les instructions.
- Finalement, vous serez invité à commencer l'évaluation comprenant 12 tests d'environ 3 minutes chaque.

3) Le suivi téléphonique (5 minutes)

- Un membre de l'équipe de recherche fera un suivi téléphonique de 5 minutes à tous les 3 mois, jusqu'à un an après l'évaluation psychométrique.
- Un questionnaire sur votre état de santé vous sera administré au téléphone.

RISQUES ET INCONVÉNIENTS

Il n'y a aucun risque physique connu à participer à ce projet de recherche.

AVANTAGES

Il se peut que vous retiriez un bénéfice personnel de votre participation à ce projet de recherche, mais on ne peut vous l'assurer.

CONFIDENTIALITÉ

Durant votre participation à ce projet de recherche, le chercheur responsable de ce projet ainsi que les membres de son personnel de recherche recueilleront, dans un dossier de recherche, les renseignements vous concernant et nécessaires pour répondre aux objectifs scientifiques de ce projet de recherche.

Ces renseignements peuvent comprendre les informations contenues dans votre dossier médical, concernant votre état de santé passé et présent, vos habitudes de vie ainsi que les résultats de tous les tests, examens et procédures qui seront réalisés. Votre dossier peut aussi comprendre d'autres renseignements tels que votre nom, votre sexe, votre date de naissance et votre origine ethnique.

Tous les renseignements recueillis demeureront confidentiels dans les limites prévues par la loi. Vous ne serez identifié(e) que par un numéro de code. La clé du code reliant votre nom à votre dossier de recherche sera conservée par le chercheur responsable de ce projet de recherche.

Ces données de recherche seront conservées pendant au moins 10 ans par le chercheur responsable de ce projet de recherche.

Les données de recherche pourront être publiées ou faire l'objet de discussions scientifiques, mais il ne sera pas possible de vous identifier.

À des fins de surveillance et de contrôle, votre dossier de recherche ainsi que vos dossiers médicaux pourront être consultés par une personne mandatée par des organismes réglementaires, au Canada, tel que Santé Canada, de l'établissement ou du comité d'éthique de la recherche. Ces personnes et ces organismes adhèrent à une politique de confidentialité.

Vous avez le droit de consulter votre dossier de recherche pour vérifier les renseignements recueillis et les faire rectifier au besoin.

COMMUNICATION DES RÉSULTATS GÉNÉRAUX

Vous pourrez connaître les résultats généraux de cette étude si vous en faites la demande au chercheur responsable à la fin de l'étude.

POSSIBILITÉ DE COMMERCIALISATION

Votre participation au projet de recherche pourrait mener à la création de produits commerciaux qui pourraient être éventuellement protégés par voie de brevet ou autres droits de propriété intellectuelle. Cependant, dans un tel cas, vous ne pourrez en retirer aucun avantage financier.

FINANCEMENT DU PROJET

Le chercheur responsable du projet utilise ses fonds de recherche pour mener à bien cette étude.

COMPENSATION

Vous ne recevrez pas de compensation financière pour votre participation à ce projet de recherche.

EN CAS DE PRÉJUDICE

Si vous deviez subir quelque préjudice que ce soit par suite de toute procédure reliée à ce projet de recherche, vous recevrez tous les soins et services requis par votre état de santé.

En acceptant de participer à ce projet de recherche, vous ne renoncez à aucun de vos droits et vous ne libérez pas le chercheur responsable de ce projet de recherche, l'organisme subventionnaire et l'établissement de leur responsabilité civile et professionnelle.

PARTICIPATION VOLONTAIRE ET DROIT DE RETRAIT

Votre participation à ce projet de recherche est volontaire. Vous êtes donc libre de refuser d'y participer. Vous pouvez également vous retirer de ce projet à n'importe quel moment, sans avoir à donner de raisons, en informant l'équipe de recherche.

Votre décision de ne pas participer à ce projet de recherche ou de vous en retirer n'aura aucune conséquence sur la qualité des soins et des services auxquels vous avez droit ou sur votre relation avec les équipes qui les dispensent.

Le chercheur responsable de ce projet de recherche, le comité d'éthique de la recherche peut mettre fin à votre participation, sans votre consentement. Cela peut se produire si de nouvelles découvertes ou informations indiquent que votre participation au projet n'est plus dans votre intérêt, si vous ne respectez pas les consignes du projet de recherche ou encore s'il existe des raisons administratives d'abandonner le projet.

Si vous vous retirez du projet ou êtes retiré(e) du projet, l'information et le matériel déjà recueillis dans le cadre de ce projet seront néanmoins conservés, analysés ou utilisés pour assurer l'intégrité du projet.

Toute nouvelle connaissance acquise durant le déroulement du projet qui pourrait avoir un impact sur votre décision de continuer à participer à ce projet vous sera communiquée rapidement.

IDENTIFICATION DES PERSONNES-RESSOURCES

Si vous avez des questions ou éprouvez des problèmes en lien avec le projet de recherche, ou si vous souhaitez vous en retirer, vous pouvez communiquer avec le chercheur responsable, Christopher Rose, ou avec une personne de l'équipe de recherche au numéro suivant: 514-890-8000-35739 (entre 8h00 et 17h00, du lundi au vendredi).

Pour toute question concernant vos droits en tant que participant(e) à ce projet de recherche ou si vous avez des plaintes ou des commentaires à formuler, vous pouvez communiquer avec le commissaire local aux plaintes et à la qualité des services du CHUM, au numéro 514-890-8484.

SIGNATURE

J'ai pris connaissance du formulaire d'information et de consentement. On m'a expliqué le projet de recherche et le présent formulaire d'information et de consentement. On a répondu à mes questions et on m'a laissé le temps voulu pour prendre une décision. Après réflexion, je consens à participer à ce projet de recherche aux conditions qui y sont énoncées.

J'autorise l'équipe de recherche à avoir accès à mon dossier médical.
J'autorise le chercheur ou son équipe à informer mon médecin traitant de ma participation à ce projet et à lui transmettre toute information pertinente:

◯ Oui
◯ Non

Nom et coordonnées du médecin traitant:

_____

Votre nom:

_____

Votre signature:

_____

Date:

_____

SECTION RÉSERVÉE À L'ÉQUIPE DE RECHERCHE

SIGNATURE DE LA PERSONNE QUI OBTIENT LE CONSENTEMENT

J'ai expliqué au/à la participant(e) le projet de recherche et le présent formulaire d'information et de consentement et j'ai répondu aux questions qu'il/elle m'a posées.

Nom : Silícia Ane Tres

Signature :

Date:

_____

ENGAGEMENT DU CHERCHEUR RESPONSABLE

Je certifie qu'on a expliqué au/à la participant(e) le présent formulaire d'information et de consentement, que l'on a répondu aux questions que le sujet de recherche avait.
Je m'engage, avec l'équipe de recherche, à respecter ce qui a été convenu au formulaire d'information et de consentement et à en remettre une copie signée et datée au/à la participant(e).

Nom: Christopher Rose

Signature:

Annex 3. Recruitment Letter

CRCHUM
CENTRE DE RECHERCHE
Centre hospitalier
de l'Université de Montréal

Université de Montréal

Objet : Recrutement au projet de recherche *Tests psychométriques chez des patients cirrhotiques*

Madame, Monsieur,

La présente est pour vous faire part de la possibilité de participer à un projet de recherche clinique mené par le Centre de recherche du CHUM, en collaboration avec le service d'hépatologie du CHUM.

L'étude consiste en l'évaluation de 12 tests potentiels pour le diagnostic de l'encéphalopathie hépatique, une complication fréquente de la cirrhose. Ce sont 12 courts tests sous forme de jeux vidéo interactifs en ligne et qui évaluent différentes fonctions mentales. L'évaluation dure entre 35 et 45 minutes.

Un membre de notre équipe de recherche vous contactera par téléphone afin de confirmer votre intérêt à participer au projet de recherche. Si vous désirez prendre contact avant, vous pouvez nous envoyer un courriel au projet.cbs@hepato-neuro.ca ou nous appeler au 514 890-8000, poste 23607 du lundi au vendredi de 9h à 17h. Pour avoir plus d'informations, vous pouvez également visiter notre site web à l'adresse https://hepato-neuro.ca/fr/labo/recrutement/

Notez bien que votre décision de participer ou non au projet de recherche n'entraînera aucune conséquence dans votre suivi clinique non plus que dans vos relations avec votre médecin traitant ou son équipe.

Je vous prie d'agréer mes plus sincères salutations,

Catherine Vincent, MD
Chef du service d'hépatologie du CHUM

Direction de la recherche
Axe Cardiométabolique
Téléphone : 514 890-8000
www.crchum.com

Pavillon R
900, rue Saint-Denis
Montréal (Québec)
H2X 0A9

Pavillon S
850, rue Saint-Denis
Montréal (Québec)
H2X 0A9

Annex 4. Recruitment Folder

LABORATOIRE HÉPATO-NEURO

Qui sommes-nous?

Notre laboratoire Hépato-Neuro fait partie du Centre de recherche du CHUM, affilié à l'Université de Montréal.

Nous avons à cœur la santé des patients cirrhotiques. Nous menons des études de recherche ayant pour but de comprendre la relation entre le foie et le cerveau. Nous travaillons à identifier et prévenir les complications de la cirrhose afin d'optimiser la qualité de vie des patients atteints de la cirrhose.

Directeurs du laboratoire

Christopher Rose, Ph. D.
Professeur titulaire, Département de médecine, Université de Montréal
Chercheur au CRCHUM

Chantal Bémeur, Ph. D., Dt. P.
Professeure agrégée, Département de nutrition, Université de Montréal
Chercheuse au CRCHUM

Le lien entre le foie et le cerveau vous intéresse?

Participez à notre étude en ligne avec des jeux vidéos!

CRCHUM CENTRE DE RECHERCHE

LABO HÉPATO NEURO

OBJECTIF DU PROJET

Ce projet de recherche consiste à étudier le lien entre le foie et le cerveau par le biais de tests psychométriques.

Un test psychométrique est une évaluation objective d'un trait psychologique comme par exemple la mémoire, la concentration, l'aptitude, etc. Des tests psychométriques en ligne ont été élaborés. Ils sont abordables, faciles d'utilisation et ne prennent que 35 à 45 minutes comparativement aux tests traditionnels sur papier et stylo d'une durée de 2 à 3 heures.

Le but de cette étude est de valider les tests psychométriques disponibles en ligne chez les patients cirrhotiques.

NATURE DE LA PARTICIPATION

Cette étude comporte 3 phases :

Phase de détermination d'éligibilité (Durée : 15 minutes)

Une personne de notre équipe communiquera avec vous au téléphone. Elle vous expliquera en détail le projet de recherche, vérifiera votre intention de participer à ce projet de recherche ainsi que les critères d'éligibilité.

Phase d'évaluation en ligne

(Durée : 35 à 45 minutes)

L'équipe de recherche vous enverra un lien par courriel qui vous donne accès à l'évaluation en ligne. Il s'agit de tests sous forme de jeux vidéo interactifs. Vous serez installé chez vous dans une pièce fermée. Cet environnement confidentiel et tranquille permettra de vous concentrer afin de répondre aux questions et de faire les tests.

Phase de suivi

(Durée : 5 minutes)

L'équipe de recherche fera un suivi téléphonique de 5 minutes à tous les 3 mois, jusqu'à un an après l'évaluation psychométrique.

CONTACTEZ-NOUS

Pour avoir plus d'informations ou pour participer :

514 890-8000, poste 23607
projet.cbs@hepato-neuro.ca
900, rue Saint-Denis, Montréal, H2X 0A9

www.hepato-neuro.ca

Le projet de recherche a été approuvé par le comité d'éthique du CHUM

CRCHUM CENTRE DE RECHERCHE

LABO HÉPATO NEURO

Annex 5. Project page on the Hepato- Neuro Lab website

This project is intended only for cirrhotic patients followed at the CHUM.

Play for research!

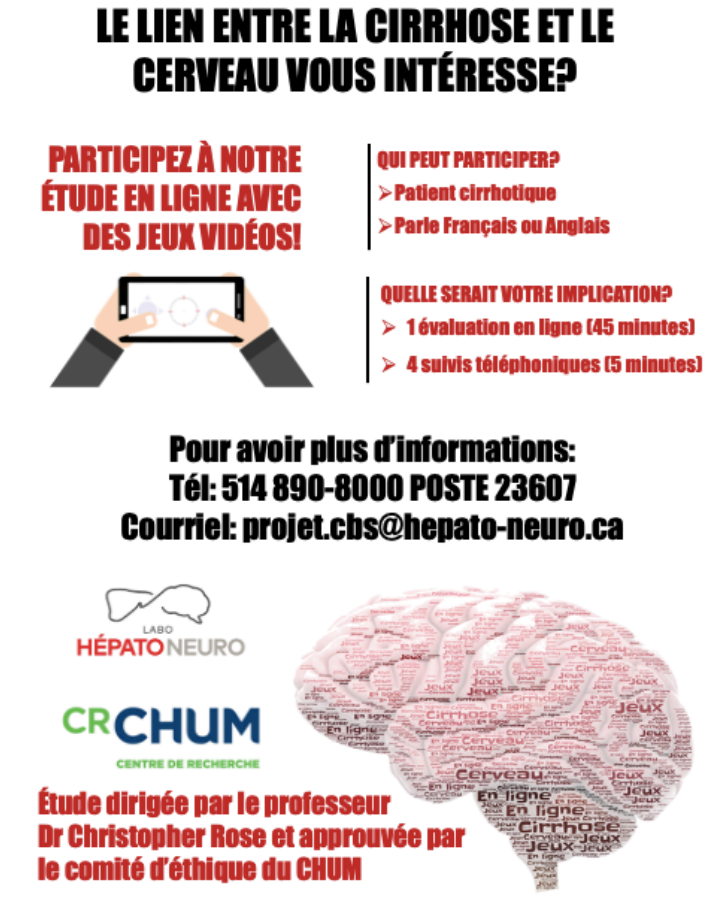
If you have cirrhosis and a computer or a tablet with an internet connection, you can participate in the project from home!

The aim is to evaluate **online tests** for the diagnosis of hepatic encephalopathy, a common and potentially treatable complication of cirrhosis. The tests are small games (with their instructions), which assess different mental areas related to cirrhosis.

Your involvement consists of an online game session (45 minutes), in addition to a phone call (5 minutes) every 3 months for 1 year to monitor your health condition.

If you are interested, or for more information, contact Silícia Ane Tres!

514-890-8000 poste 23607
projet.cbs@hepato-neuro.ca



Annex 6. Beginning of study: email confirmation and access to the information and consent form

Titre: Projet CBS – CRCHUM – Formulaire d'information et de consentement (FIC)

Bonjour [appellation][nom],

Merci d'avoir manifesté un intérêt à participer à notre étude. Bienvenu (e) au projet CBS!

Tel que mentionné lors de notre conversation téléphonique, vous recevez ce message contenant le **formulaire d'information et de consentement (FIC).** Ce formulaire récapitule toutes les informations concernant votre participation au projet. Il est important que de le lire attentivement et de bien comprendre chaque partie du formulaire.

Veuillez noter que votre consentement est essentiel pour participer à un projet de recherche Ainsi, vous devez signer le FIC en écrivant votre nom et la date aux endroits indiqués.

Votre participation est volontaire et vous pouvez également vous retirer à tout moment, en informant l'équipe de recherche par téléphone ou par courriel.

Étapes à suivre pour donner votre consentement à participer au projet:

1. Pour accéder au formulaire d'information et consentement, cliquez sur le lien suivant: FIC
2. Lire le formulaire
3. Signer et dater le formulaire
4. Cliquer sur « envoyer »

Merci pour votre participation et n'hésitez pas à communiquer si vous avez des questions.

Cordialement,

Projet « Tests psychométriques chez les patients cirrhotiques- CBS »
Centre de Recherche du CHUM
projet.cbs@hepato-neuro.ca
514 890 8000, poste 23607

Annex 7. Email notification in case of absence of signed consent after 20 days of verbal consent

Titre : Projet CBS – Avis d'absence de réception de votre consentement

Bonjour [appellation][nom],

Ce message vise à vous informer qu'à ce jour, nous n'avons pas reçu votre consentement concernant la participation au projet de recherche intitulé "Tests psychométriques chez des patients cirrhotiques - CBS" du CR CHUM.

Veuillez noter que votre consentement est essentiel pour participer à un projet de recherche et que, sans lui, vous ne pourrez pas y participer.
Votre participation est volontaire et vous pouvez également vous retirer à tout moment, en informant l'équipe par téléphone ou courriel.

Étapes à suivre pour donner votre consentement à participer au projet:

1. Pour accéder au formulaire d'information et consentement, cliquez sur le lien suivant: FIC
2. Lire le formulaire
3. Signer e dater le formulaire
4. Cliquer en « envoyer »

Merci pour votre participation et n'hésitez pas à communiquer si vous avez des questions.

Cordialement,

Projet CBS
Labo Hépato- Neuro_ Centre de Recherche du CHUM
projet.cbs@hepato-neuro.ca
514 890 8000, poste 23607

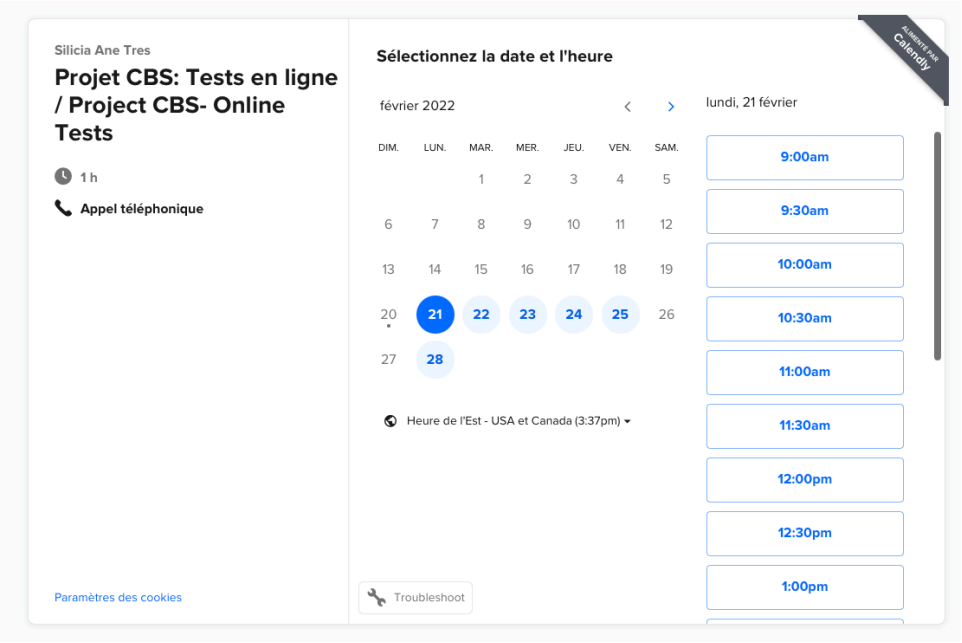
Annex 8. Confirmation message after obtaining the consent form signature

Bonjour [appellation][nom],

Merci d'avoir pris le temps de remplir le formulaire!
Ce message vise à vous informer que nous avons bien reçu votre consentement concernant la participation au projet de recherche intitulé "Tests psychométriques chez des patients cirrhotiques - CBS" du CRCHUM.
Veuillez choisir la date et l'heure qui vous conviennent pour effectuer les tests en ligne en cliquant sur le calendrier suivant: https://calendly.com/silicia-ane-tres/tests-en-ligne
Vous recevrez un appel à l'heure choisie pour vous donner des instructions, ainsi qu'un courriel avec le lien pour accéder aux tests.
Merci encore pour votre participation!
Cordialement,

Projet CBS
Labo Hépato-Neuro, Centre de Recherche du CHUM
projet.cbs@hepato-neuro.ca
514 890 8000, poste 23607

Annex 9. Online calendar to select the day and time for the cognitive tests



Annex 10. Email with a copy of the signed information and consent form

Bonjour [appellation][nom],

Dans ce message, vous recevez une copie du formulaire d'information et de consentement que vous avez rempli concernant votre participation au projet de recherche intitulé "Tests psychométriques chez des patients cirrhotiques - CBS" du CRCHUM.
Si vous n'avez pas encore sélectionné la date et l'heure de vos tests en ligne, vous pouvez choisir en cliquant sur le lien suivant: Prise de rendez-vous.
Si vous avez déjà choisi une date, aucune action n'est requise de votre part. Vous recevrez un appel à l'heure choisie pour vous donner des instructions pour le test.
Merci pour votre participation!

Cordialement,

Projet CBS
Labo hépato-neuro, Centre de Recherche du CHUM
projet.cbs@hepato-neuro.ca
514 890 8000, poste 23607

Annex 11. Access to the CBS online cognitive assessment by email

Titre : Projet CBS – Lien pour votre évaluation en ligne

Bonjour [appellation][nom],

Dans ce message vous trouverez les instructions pour réaliser l'évaluation en ligne dans le cadre du projet de recherche intitulé "Tests psychométriques chez des patients cirrhotiques - CBS" du CR CHUM.

Cette évaluation comprend 12 tests développés et fournis par la plate-forme en ligne **Cambridge Brain Sciences (CBS).** Elle dure entre 35 et 45 minutes. Ces tests sont des jeux qui évaluent la mémoire, la concentration, le raisonnement, et autres.

Informations concernant votre préparation pour la réalisation des tests :

- **Vous devez choisir un endroit calme où vous pouvez effectuer les tests sans interruption**
- **Vous devez effectuer les tests vous-même**
- **Vous devez être reposé(e) pour pouvoir vous concentrer pendant le test**
- **Vous devez lire attentivement les instructions pour chaque test et entrainez-vous avant de passer le test**
- **Si vous faites des erreurs, ne vous inquiétez pas. L'important est que vous interagissiez avec attention, selon les instructions données pour chaque test et que vous terminiez les 12 tests**

Informations concernant le fonctionnement de la plateforme des tests en ligne :

- **Les tests doivent être effectués en un seul accès, il suffit donc de cliquer sur le lien lorsque vous êtes prêt à le réaliser. Ce lien est unique et personnalisé, vous ne devez pas le partager.**
- **Il sera possible de faire une courte pause entre les tests si nécessaire, et de les poursuivre par la suite, sans fermer la fenêtre du navigateur**
- **Vous pouvez effectuer les tests depuis un ordinateur ou une tablette (les téléphones mobiles ne sont pas pris en charge)**

Étapes à suivre:

1. Pour accéder aux tests en ligne, cliquez sur le lien suivant: CBS
2. Choisir la langue (Français ou Anglais)
3. Suivre les instructions

Merci pour votre participation et n'hésitez pas à communiquer si vous avez des questions.

Cordialement,

Projet CBS
Labo Hépato- Neuro_ Centre de Recherche du CHUM
projet.cbs@hepato-neuro.ca
514 890 8000, poste 236

Annex 12. E-mail notification of non-receipt of the patient's online assessment

Titre : Projet CBS – Avis d'absence de réception de votre évaluation en ligne

Bonjour [appellation][nom],

Ce message vise à vous informer qu'à ce jour, nous n'avons pas encore reçu votre évaluation concernant la participation au projet de recherche intitulé "Tests psychométriques chez des patients cirrhotiques - CBS" du CR CHUM.

Vous pouvez toujours accéder à l'évaluation en ligne en suivant les étapes ci-après. Ceci ne vous prendra que 45 minutes au maximum.

Étapes à suivre:

ATTENTION : CLIQUEZ SUR LE LIEN SUIVANT UNIQUEMENT LORSQUE VOUS ÊTES PRÊT(E) À FAIRE LES TESTS.

1. Pour accéder aux tests en ligne, cliquez sur le lien suivant: CBS
2. Choisir la langue (Français ou Anglais)
3. Suivre les instructions

Merci pour votre participation et n'hésitez pas à communiquer si vous avez des questions.

Cordialement,

Projet CBS
Labo Hépato- Neuro_ Centre de Recherche du CHUM
projet.cbs@hepato-neuro.ca
514 890 8000, poste 23607

Annex 13. Confirmation message after tests are completed

Titre : Projet CBS – Accusé de réception de résultats des tests en ligne

Bonjour Madame/ Monsieur (*nom du patient*),

Ce message vise à vous informer que nous avons bien reçu votre évaluation en ligne concernant les tests pour la participation au projet de recherche. Merci!

Votre évaluation est terminée.

Vous recevrez désormais des appels tous les 3 mois pour surveiller votre état de santé et vérifier les informations liées à la cirrhose, telles que l'apparition ou modification des symptômes, utilisation de médicaments, survenue d'hospitalisations et autres. Vous répondrez à un court questionnaire de suivi d'une durée moyenne de 5 minutes.

Vous recevrez un courriel une semaine avant l'appel téléphonique pour confirmer la date et heure.

Si vous avez choisi de recevoir des SMS, vous recevrez également un SMS par téléphone 1 jour avant l'appel téléphonique.

Merci pour votre participation et n'hésitez pas à communiquer si vous avez des questions.

Cordialement,

Projet CBS
Labo Hépato- Neuro_ Centre de Recherche du CHUM
projet.cbs@hepato-neuro.ca
514 890 8000, poste 23607

Annex 14. Message before follow-up calls

Titre : Projet CBS – Suivi (insérer la date)

Bonjour Madame/ Monsieur (*nom du patient*),

Ce message vise à vous informer que nous effectuerons un suivi téléphonique (insérer la date) pour évaluer votre état de santé des 3 derniers mois.

Nous vous rappelons que le but de cet appel est de surveiller votre état de santé et vérifier les informations liées à la cirrhose, telles que l'apparition ou modification des symptômes, l'utilisation de médicaments, la survenue d'hospitalisations (principalement liés au développement d'un épisode d'encéphalopathie hépatique) et autres.

Vous répondrez à un court questionnaire de suivi d'une durée moyenne de 5- 10 minutes.

Merci pour votre participation et n'hésitez pas à communiquer si vous avez des questions.

Cordialement,

Projet CBS
Labo Hépato- Neuro_ Centre de Recherche du CHUM
projet.cbs@hepato-neuro.ca
514 890 8000, poste 23607

Annex 15. Follow-up questionnaire

Questionnaire de suivi téléphonique

En vous référant à la période correspondant aux trois derniers mois,

1. Avez-vous remarqué un changement dans votre rythme de sommeil?

   ( ) Somnolence diurne

   ( ) Insomnie

   ( ) Changement du rythme de sommeil non spécifié

   ( ) Aucun changement

2. Avez-vous eu des sauts d'humeur?

   ( ) Oui ( ) Non

3. Avez-vous expérimenté des périodes de perte de mémoire?

   ( ) Oui ( ) Non

4. Avez-vous consommé de l'alcool en excès?

   ( ) Oui ( ) Non

5. Avez-vous remarqué une diminution de l'attention portée aux activités quotidiennes/ au travail ?

   ( ) Oui ( ) Non

6. Avez- vous fait des chutes?

   ( ) Oui ( ) Non

7. Avez-vous été impliqué dans un accident de voiture? (si le patient conduit)

   ( ) Oui ( ) Non

8. Y a-t-il eu un changement dans vos médicaments ?

   ( ) Oui ( ) Non

   Si oui, prenez-vous un ou plusieurs types de médicaments suivants?

   ( ) Bénzodiazépines

   ( ) Autres médicaments pyschotropiques

   ( ) Antibiotiques

   ( ) Lactulose/ Rifaximine

   ( ) Inhibiteurs de la pompe à protons

   ( ) Diurétiques

( ) Probiotiques

( ) Beta bloquants

( ) Autres, Précisez _____

9. Avez-vous présenté un ou plusieurs des symptômes suivants?

( ) Fièvre

( ) Augmentation du volume de l'abdomen (ascite)

( ) Constipation intestinale

( ) Sang dans vos selles

( ) Selles anormalement plus noires que d'habitude (méléna)

( ) Vomissements avec du sang

10. Avez-vous été hospitalisé au cours de cette période?

( ) Oui ( ) Non

a. À quel hôpital avez-vous été hospitalisé? _____

b. Raisons liées à l'hospitalisation

( ) Encéphalopathie hépatique

( ) Saignement gastro-intestinal

( ) Shunt intrahépatique par voie transjugulaire (TIPS)

( ) Sevrage de l'alcool

( ) Autre, Précisez : _____

Annex 16. End of study: email confirmation

Titre : Projet CBS – fin d'étude

Bonjour Madame/ Monsieur (*nom du patient*),

Ce message a pour but de vous informer que votre participation à l'étude « Tests psychométriques sur les patients cirrhotiques - CBS » est terminée. Merci!

Nous sommes très reconnaissants de votre participation et espérons pouvoir contribuer avec les résultats de cette étude pour le meilleur traitement des patients atteints de cirrhose, grâce à la collaboration de patients comme vous.

Au nom de toute l'équipe de recherche du labo Hépato - neuro au CRCHUM, nous vous souhaitons une bonne continuité dans votre traitement et nous vous remercions de votre participation.

Cordialement,

Projet CBS
Labo Hépato- Neuro_ Centre de Recherche du CHUM
projet.cbs@hepato-neuro.ca
514 890 8000, poste 23607