New Methods of Testing and Brain Imaging in Hepatic Encephalopathy: A Review

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KEYWORDS
- Hepatic encephalopathy • Ammonia • Psychometric testing • Stroop app
- Brain imaging • Magnetic resonance spectroscopy • Single-photon emission CT
- PET

KEY POINTS
- Hepatic encephalopathy (HE) is a clinical diagnosis requiring the exclusion of other causes of altered cerebral function.
- Diagnosis requires the presence of decompensated cirrhosis, acute liver failure, acute-on-chronic liver failure, or portosystemic shunting without cirrhosis.
- Psychometric tests are useful in the diagnosis of covert HE (CHE) but can be expensive and time consuming.
- Serum ammonia measurement is not routinely recommended for diagnosis.
- Functional brain imaging plays an important role in the diagnosis and understanding the pathogenesis of HE.

INTRODUCTION

HE comprises a spectrum of neuropsychiatric manifestations that can occur in patients with cirrhosis, acute liver failure, acute-on-chronic liver failure, or major portosystemic shunting without intrinsic liver disease. It is characterized by disturbances in cognitive and motor function that can manifest as a change in personality, altered mood, diminished intellectual capacity, abnormal muscle tone, and tremor, among other symptoms in chronic liver disease. The manifestations of this entity in acute liver failure can include abrupt-onset delirium, seizures, and coma as a result of cerebral...
edema, increased intracranial pressure, and eventually brain herniation as a terminal event.\textsuperscript{3,4} Early symptoms of HE might be subtle in nature and require psychometric testing to be identified.\textsuperscript{5} Clinically obvious psychomotor derangements may occur later on as the disease progresses. Hence, it is imperative to identify these subtle manifestations to facilitate early diagnosis.\textsuperscript{5} A diagnosis of HE is mainly clinical and usually made by the exclusion of other causes of brain or spinal cord dysfunction and proved by response to available therapy.\textsuperscript{5} Certain imaging characteristics, such as basal ganglia hyperintensity on T1-weighted MRIs, are present in patients with end-stage liver disease, pointing toward the diagnosis, but are not pathognomonic for HE.\textsuperscript{6} Functional brain imaging is valuable and has provided important information into the understanding of the pathophysiology of HE but an optimal, clinically relevant and easily accessible test remains elusive.\textsuperscript{7}

**DIAGNOSING HEPATIC ENCEPHALOPATHY**

To suspect a diagnosis of HE, as the term implies, a clinician has to first identify the presence of cirrhosis, acute liver failure, or portosystemic shunts without intrinsic liver disease. Testing should include methods for diagnosing CHE and overt HE.\textsuperscript{5} Early symptoms include cognitive deficits in attention, visual perception, visuospatial construction, motor speed, and accuracy.\textsuperscript{8} The subtle nature of these deficits can require psychometric testing for diagnosis. Clinically obvious symptoms and signs occur later on as the disease is advancing but a diagnosis can be made only after exclusion of other causes of cerebral dysfunction.\textsuperscript{5} Additional diagnostic approaches include biochemical analysis to determine serum ammonia levels, brain imaging, electroencephalogram (EEG), lumbar puncture (LP), and other new methods of functional brain imaging, like magnetic resonance spectroscopy (MRS), PET, and single-photon emission computed tomography (SPECT).\textsuperscript{5}

**PSYCHOMETRIC TESTS**

Hamster and colleagues\textsuperscript{9} paved the way for further standardization in testing methods for CHE: 96 cirrhotic patients and 163 healthy age-matched controls were subjected to more than 30 different psychometric tests to assess cognitive domains ranging from premorbid intelligence levels to verbal abilities to visuomotor function and to coordination. It was published that the line tracing test, pegboard, aiming and steadiness of motor performance scale, and digit symbol test could effectively differentiate cirrhotic and noncirrhotic patients. CHE patients show abnormalities, particularly in areas of attention (loss of vigilance and disorientation), executive functions (problem solving, planning, and judgment), visuospatial coordination, and psychomotor speed (reaction times).\textsuperscript{10} Underlying many of these deficits is an impaired response inhibition.\textsuperscript{11} Psychometric testing strategies focus on defining abnormalities related to these domains using neuropsychological or neurophysiologic tests.\textsuperscript{11} An overall brief description of available psychometric tests and their practical application in diagnosis of CHE are depicted in Table 1.

The drawbacks of applying psychometric tests in CHE patients include time and effort added to outpatient visits, lack of standardization, reliance on psychological expertise to administer and interpret results, the expensive and copyrighted testing procedures involved in the application of these tests, and potential reimbursement issues.\textsuperscript{12}

**STROOP APPLICATION**

The Stroop smartphone application (app), which was developed by Bajaj and colleagues,\textsuperscript{13} is a short and recently validated test to screen and diagnose patients...
with CHE. It is marketed as the EncephalApp—Stroop Test (available as a free download on iTunes). The app is easy to administer and quick to teach patients, and the interpretation is simple without the need for a psychologist to administer the test. EncephalApp has an on state, which is a measure of response inhibition and motor speed, and an off state, which assesses psychomotor ability. The results measured include off time (total time required to complete 5 correct runs in the off state), on time (total time required to complete 5 correct runs in the on state), off time plus on time, and the number of runs required to complete 5 correct off and on runs by the subject. Both components were administered to subjects after 2 training runs were given for each state.

During validation of the test, all patients with cirrhosis performed worse on standard paper-and-pencil psychometric tests and EncephalApp tests compared with controls. Patients with cirrhosis and overt HE performed worse than those without overt HE. An off time plus on time value of greater than 190 seconds identified all patients with CHE. EncephalApp times also correlated with accidents and illegal turns in driving simulation tests. It was determined that the app has good face validity, test-retest reliability,
and external validity for diagnosis of CHE. Use of this app may facilitate the evaluation and treatment of patients with CHE in the United States, where testing for this stage of HE is not routinely administered.

**AMMONIA**

Measurement of serum ammonia levels may be helpful in the initial evaluation of patients with altered mental status, when the presence of significant liver disease has not been established and other causes are suspected. The diagnostic value of ammonia is limited by several factors. Levels can be influenced by potential errors associated with sample collection, handling, and storage. Artifactual increase in ammonia levels can be seen in patients with mild exertion prior to sampling and spontaneous release of ammonia from red blood cells in blood samples left at room temperature. In patients with acute liver failure, elevated levels of arterial ammonia (>150 mg/dL) have been associated with an increased risk of complications and cerebral herniation. In patients with cirrhosis, no consistent correlation between serum ammonia level, risk of cerebral edema, and severity of HE has been identified.

Blood for ammonia level should be collected from a stasis-free vein. Avoid fist clenching or application of a tourniquet, which can elevate ammonia levels due to release from skeletal muscle. Blood should be collected in a lithium heparin– or sodium heparin–containing (green top) Vacutainer because heparin inhibits the release of ammonia from red blood cells. Samples should be stored and transported in an ice bath and tested within 20 minutes for ammonia assay.

Measurement of serum ammonia levels alone might not be a definitive diagnostic tool for HE and is not indicated for routine diagnosis in daily clinical practice. Other metabolic disturbances that may affect the cerebral function, including hyponatremia in cirrhotic patients and hypoglycemia in patients with acute liver failure, need to be considered.

**BRAIN IMAGING**

Clinicians working with patients suffering from HE currently have to accept that there are no specific imaging or clinical findings that ensure the diagnosis. If a patient with liver disease presents with neuropsychiatric aberrations, a diagnosis of HE has to be considered after excluding other possible causes. Therefore, in clinical practice, CT and MRI of the brain are used to exclude other causes of cerebral dysfunction, including intracranial hemorrhage, infarction, infection, or tumor. Although CT scan findings are usually unremarkable, subtle abnormalities have been observed in cirrhotic patients without HE that suggest frontal cortical atrophy and mild cerebral edema. Conventional T1 or T2 MRI is available in most centers and allows exclusion of other neurologic diseases and may reveal typical signs of HE. A majority of patients with cirrhosis or portosystemic shunts exhibit bilateral, symmetric high signal intensity at the globus pallidus and substantia nigra. This signal may increase after transjugular intrahepatic portosystemic shunt placement and reverses after occlusion of congenital portosystemic shunts. Rise in concentration of manganese, a paramagnetic substance in the central nervous system, with preferential deposition in the globus pallidus, is the most possible explanation for this finding. Although pallidal hyperintensities are found in 90% of cirrhotic patients, they are not closely linked to the presence of HE. It has been demonstrated that cirrhotic patients with no clinical or neuropsychiatric signs of HE can also show severe signal alterations, whereas others with HE may present with slight signal alterations only. Clinical experience, however, indicates that the absence of T1 high signal intensity on MRI is a strong indicator against interpreting neurologic manifestations as secondary to liver disease.
LUMBAR PUNCTURE

Unlike brain imaging, fluid analysis of cerebrospinal fluid obtained from LP is not required for diagnosis of HE but may be necessary when other conditions, such as meningitis, encephalitis, and subarachnoid hemorrhage, are suspected. Moreover, patients with liver disease may present with severe coagulopathy and decreased platelet count, and LP is often contraindicated due to risk of bleeding. In addition patients with acute liver failure might suffer from cerebral edema and increased intracranial pressure and are at a risk of brainstem herniation subsequent to the procedure. When performed to exclude other concomitances in patients with HE, however, increased levels of cerebrospinal fluid glutamine concentrations as well as an increase in aromatic amino acids have been identified.

ELECTROENCEPHALOGRAM

EEG provides useful information to quantify brain dysfunction in HE. More than 50 years ago, Parsons-Smith and colleagues showed that EEG patterns have an approximate relationship with behavioral features of HE. The first EEG sign of HE is a low-frequency alpha rhythm disturbed by random waves in the theta region over both cerebral hemispheres. Increases in the severity of HE induce progressive increases in the theta band activity along with high-voltage delta band activity. Triphasic waves can be seen at this stage although they are not specific to HE and can be observed in other types of metabolic encephalopathy. In comatose patients, the tracings are formed by high-voltage arrhythmic delta waves and finally a flat EEG. Once a flat EEG is reached, further information of brain activity can be obtained by somatosensory evoked potentials. EEG quantification provides good assessment of risk for developing overt HE and mortality at 1-year follow-up in patients who do not display symptoms of overt encephalopathy at the time of examination. EEG, thus, is a valuable tool in clinical practice to investigate HE. Although to a lesser extent than clinical grading of HE, inter- and intraobserver variability does exist with EEG. With cartography of cerebral electrical activity, or brain mapping, EEG can analyze various regions of the brain. This technique has high sensitivity for functional alterations of the brain, and 85% of patients with no clinical symptoms of HE show abnormalities on this technique. This technology also serves to simplify the interpretation of EEG.

The interpretation of EEG findings is independent of patient’s cooperation and education level. On the contrary, psychometric testing is influenced by patient education and cooperation. EEG might be considered a complementary test. In CHE, it has proved to have a higher predictive value on survival compared with psychometric testing. EEG can objectively depict changes in brain function after treatment and can be a useful follow-up test to assess treatment response. It has been reported that there is prognostic value of the EEG findings for the development of HE and mortality in cirrhotic patients. Marked improvement in EEG findings has also been demonstrated in the post-transplant setting and may play a role in the evaluation of post-transplant neurologic complications.

MAGNETIC RESONANCE SPECTROSCOPY

MRS has been widely used in recent times for the evaluation of HE as a result of improved sequence development and higher field strength to resolve metabolite signals. The technique is based on the same physical principles as MRI and the detection of energy exchange between external magnetic fields and specific nuclei within atoms. MRS can be performed with existing MRI equipment and modified with
additional software and hardware. It is considered investigational and not routinely available for clinical use.

MRS facilitates the investigation of HE and effects of increased ammonia supply and metabolism in the cerebral tissue in vivo at the molecular level.\(^6\) MRS detects the relaxation properties of some atoms in strong magnetic fields and can generate high-resolution images or spectrum of several metabolites that contain the atoms studied.\(^48\) A series of metabolites is displayed as peaks at different frequencies in the spectrum. Hydrogen proton-MRS demonstrates relative to creatinine an increase in glutamine/glutamate (Glx) signal and a decrease of choline (cho)-containing compounds and myo-inositol.\(^49\) Abnormalities in the Glx signal have been interpreted as an increase in brain glutamine secondary to metabolism of ammonia in the astrocyte. Disturbances of cho and myo-inositol have been interpreted as a compensatory response to increase in intracellular osmolality caused by accumulation of glutamine in astrocytes.\(^48\)

The severity of changes seen in MRS have been proposed as a signature of HE based on studies.\(^50\) In patients with cirrhosis subjected to ammonia load, hydrogen proton-MRS consistently showed increase in Glx signal accompanied by myo-inositol depletion and decrease in the cho signal in all regions.\(^51-53\) These changes are considered to reflect basic metabolic alterations of the brain in cirrhotics that are involved in the development of HE.\(^54\) In a study performed in patients with minimal HE, a decrease in myo-inositol was the most accurate predictor of minimal HE compared with MRI or psychometric tests.\(^55\) The evolution of hydrogen proton-MRS abnormalities after liver transplantation and their reversibility have been assessed in longitudinal studies.\(^30,31\) This reversibility precedes the disappearance of pallidal hyperintensity after liver transplantation and correlates with clinical neurologic improvement.\(^56\)

The extent of MRS changes increase with increasing grade of encephalopathy.\(^57\) These changes have also been observed in cirrhotic patients, with neither clinical nor psychomotor signs of cerebral dysfunction.\(^48\) Patients with Child class C cirrhosis with and without HE did not differ with regard to findings on MRS.\(^58\) The characteristic MRS changes seem to reflect metabolic alterations more than functional alterations of the brain and are not perfect indicators of HE. Therefore, MRS alterations cannot be used as a sole test to diagnose HE.

**SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY**

SPECT studies the spatial distribution of radioactive tracer technetium Tc 99 and its local metabolism in the brain. Because these radionuclides are uncommon to human body, the tracer binding or metabolism may not be identical to that of native molecule and, hence, difficulties in the interpretation of results can occur.\(^59\) Previous studies have shown increased blood flow in the basal ganglia of patients with minimal HE, suggesting increased ammonia delivery to these areas, resulting in astrocyte dysfunction and cognitive impairment.\(^60,61\) SPECT provides only relative measurements of radioactivity and allows the comparison of physiologic parameters like blood flow in different areas of the brain.

Compared with PET, discussed later, this method is more easily available and less expensive. PET is preferred in functional imaging studies of the brain, however, due to its superior spatial resolution.\(^62\) Studies that have investigated SPECT in HE have been limited by small study sizes, limiting the use of this tool in diagnosis of HE.

**PET**

PET is a nuclear imaging test based on the detection of positron emission associated with isotope decay. The 4 main isotopes used for clinical purposes are oxygen
Using these isotopes, a large number of tracers can be synthesized to study brain function. In patients with cirrhosis and HE, the tracers used $^{15}$O-$\text{H}_2\text{O}$, $^{13}$N-ammonia (NH$_3$), and $^{18}$F-fluorodeoxyglucose (FDG). PET has the ability to obtain quantitative data of isotope distribution and use the data with measurements of radioactivity in the blood to calculate physiologic parameters like blood flow, glucose metabolism, and ammonia metabolism. PET has been applied to investigate the cerebral ammonia metabolism in parallel with cerebral glucose utilization. In this study, plasma ammonia levels correlated with ammonia metabolism of the brain and with MRS in white matter. MRS also showed a correlation with cerebral glucose utilization. Ammonia metabolism and glucose utilization were, however, not associated. The study suggests that cerebral ammonia metabolism is important in the development of HE but is not the only factor.

Oxygen consumption and cerebral blood flow have been investigated in cirrhotic patients with and without HE and compared with healthy controls. A decrease in oxygen consumption and cerebral blood flow was induced by HE. It has been proposed that the inability to use delivered oxygen of patients with HE relates to a specific inhibition associated with oxidative metabolism in mitochondria. Lockwood and colleagues have used FDG-PET to investigate functional changes in HE. They were able to demonstrate a reduction in glucose metabolism in the anterior cingulate gyrus, which may reflect attention deficit found in HE patients on neuropsychiatric testing and in functional magnetic resonance studies. Alteration in cerebral blood flow has also been established using FDG-PET and $^{15}$O-PET, where poor neuropsychiatric test performance correlated with reduced blood flow in all cortical areas. Temporal lobe blood flow was found most discriminatory between HE patients and healthy volunteers.

A disadvantage of PET is its limited spatial resolution and the lack of information about the anatomic structures represented by the detected metabolic data. These disadvantages can be overcome by coregistration of PET images and MRIs of a patient and defining regions of interest, based on anatomic information provided by MRI before further analysis of the PET data. Possible clinical implications of PET is in the differential diagnosis with other neurologic disorders, like Alzheimer or Parkinson disease, for which specific radioligands are available.

PET and SPECT have provided valuable insight into the pathogenesis of HE but are not widely available diagnostic tools. Their use in the evaluation of HE is limited by the expense and availability of the tests and currently limited to research based academic centers. Promising functional data are emerging, but standardization in regard to uniformity of study protocols, imaging sequences, and analysis methods is required before they become widely available diagnostic tools for HE, which is a common but under-recognized complication of cirrhosis.

SUMMARY

Despite several advances in diagnostic testing of HE, it remains a clinical diagnosis based on clinical criteria that classify HE into various grades ranging from normal mental status to coma. Because this clinical classification is somewhat subjective, additional diagnostic tools are required. Subtle memory and attention deficits in cirrhotics are not always caused by HE and adequate diagnostic tests are required. Although ammonia levels are routinely measured in patients with altered consciousness, elevated levels do not always result in HE even in the presence of cirrhosis. Further testing is required to clarify the diagnosis and exclude other causes. Psychometric tests are used to diagnose minimal HE, although correction may be required for age and educational level and they are not routinely used in clinical practice. The
Stroop smartphone app is a short and validated psychometric test that recently has become available to assist in identifying patients with CHE. Brain imaging is used mainly to rule out other causes; although some MRI findings are characteristic in patients with cirrhosis, they are not pathognomonic of HE.

New neuroimaging techniques, especially functional brain imaging, have seen rapid development in recent years, and the data obtained from the brains of patients with different stages of liver disease have provided a better understanding of the pathogenesis. It is now known that the pattern of cerebral dysfunction in HE is restricted to certain brain anatomic structures and functional circuits involving specific metabolites, at least in the early stages of the disease. Although these tests are not standardized in terms of applying them to routine clinical practice, extensive information obtained from the use of these modalities supports their use in monitoring and evaluating the effect of current and new therapeutic agents for HE, and they may become standard of care in the near future.

REFERENCES