
Università degli Studi di Padova
Scuola di Medicina e Chirurgia
Corso di Laurea in Medicina e Chirurgia
Dipartimento di Medicina DIMED

TESI DI LAUREA

# AI-Based Identification of Artifact-Free Wake EEG Segments for Spectral Analysis in Hepatic Encephalopathy

Relatrice: Prof.ssa Sara Montagnese

Correlatrice: Dott.ssa Chiara Mangini

Laureando: Sebastiano Noriller

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## ABSTRACT

**Introduction:** Hepatic encephalopathy is a serious complication of liver disease and/or portosystemic shunting, associated with high morbidity and mortality. Wake electroencephalography (EEG) is commonly used to support its diagnosis and prognosis by detecting slowing of brain activity. However, quantitative EEG analysis still depends on manual selection of artifact-free segments, which limits its use in routine clinical practice.

**Aim of the study:** The aim of this study was to develop a machine learning-based pipeline capable of automatically selecting one-minute artifact-free wake EEG segments for subsequent automated spectral analysis.

**Materials and methods:** A Python-MATLAB workflow was developed to process approximately 12,000 archived EEG recordings stored in proprietary TRC format. A clinically annotated database including 578 evaluations with corresponding EEG recordings, clinical, psychometric, and laboratory data was refined. For each EEG included in the dataset, a one-minute artifact-free wake segment previously identified by expert clinicians was used as a reference standard. On a subset of 4 EEG recordings, a preliminary validation of the automated pipeline was performed. Spectral analysis was automatically applied to the manually selected segments to verify correct interpretation of the EEG annotations and reproducibility of spectral EEG parameters.

**Results:** A total of 578 clinical evaluations with corresponding EEG recordings and standardized diagnostic classification were included in the final dataset. A structured TRC-based EEG database was successfully constructed by linking electrophysiological recordings with confirmed clinical diagnoses. Preliminary validation of the automated EEG processing pipeline was performed on 4 EEG recordings, demonstrating good agreement between the spectral analysis implemented in the automatic algorithm and those manually performed by expert clinicians.

**Conclusion:** The results suggest that machine learning may help automate the selection of high-quality EEG segments. This may reduce variability between observers and may support the use of machine learning-based quantitative EEG analysis in the clinical assessment of hepatic encephalopathy.

## RIASSUNTO

**Introduzione:** L'encefalopatia epatica è una grave complicanza della cirrosi con/senza shunt portosistemico, ed è associata ad un'elevata morbilità e mortalità. L'elettroencefalogramma (EEG) è comunemente utilizzato come supporto diagnostico e prognostico grazie alla sua capacità di rilevare il rallentamento dell'attività cerebrale di veglia caratteristico della patologia. Tuttavia, l'analisi quantitativa dell'EEG richiede ancora la selezione manuale di segmenti privi di artefatti, limitandone l'applicazione nella pratica clinica routinaria.

**Obiettivo dello studio:** Lo scopo di questo studio è stato sviluppare una pipeline basata su tecniche di machine learning in grado di selezionare automaticamente segmenti di EEG di veglia della durata di un minuto e privi di artefatti, da destinare alla successiva analisi spettrale automatizzata.

**Materiali e metodi:** A questo scopo, è stata sviluppata una pipeline integrata Python-MATLAB per l'elaborazione di circa 12.000 registrazioni EEG archiviate nel formato proprietario TRC. È stato inoltre perfezionato un database di 578 valutazioni comprendente i tracciati EEG ed i corrispondenti dati clinici, psicometrici e laboratoristici. Per ciascun EEG incluso nel dataset è stato utilizzato come standard di riferimento un segmento di veglia di un minuto, privo di artefatti e precedentemente selezionato da clinici esperti. Su un sottoinsieme di quattro registrazioni EEG è stata effettuata una validazione preliminare della pipeline automatizzata. L'analisi spettrale è stata applicata automaticamente ai segmenti selezionati manualmente al fine di verificare la corretta interpretazione delle annotazioni EEG e la riproducibilità dei parametri spettrali.

**Risultati:** Nel dataset finale sono state incluse 578 valutazioni cliniche corredate dai rispettivi tracciati EEG e da una classificazione diagnostica standardizzata. È stato costruito con successo un database EEG strutturato basato sul formato TRC, integrando le registrazioni elettrofisiologiche con diagnosi cliniche confermate. La validazione

preliminare della pipeline automatizzata, eseguita su quattro registrazioni EEG, ha mostrato una buona concordanza tra l'analisi spettrale implementata dall'algoritmo automatico e quella di riferimento.

**Conclusioni:** I risultati ottenuti suggeriscono che l'impiego di algoritmi di machine learning possa contribuire ad automatizzare la selezione di segmenti EEG di elevata qualità, riducendo la variabilità inter-osservatore e favorendo l'integrazione dell'analisi quantitativa dell'EEG basata su machine learning nella valutazione clinica dell'encefalopatia epatica.

# 1 INTRODUCTION

## 1.1 Definition of Hepatic Encephalopathy

Hepatic encephalopathy (HE) is a frequent and severe complication of liver disease and/or portalsystemic shunt and is clinically manifested as a wide spectrum of neurological and psychiatric manifestations ranging from mild cognitive deficits to coma (1). Within the natural history of cirrhosis, the development of HE represents a critical moment in the disease progression, as it is associated with an increased risk of frequent re-hospitalizations and mortality (2).

## 1.2 Etiological classification

HE is currently classified through a multiaxial system which has been introduced in the AASLD/EASL practice guidelines and is based on four parameters (3) (Figure 1).

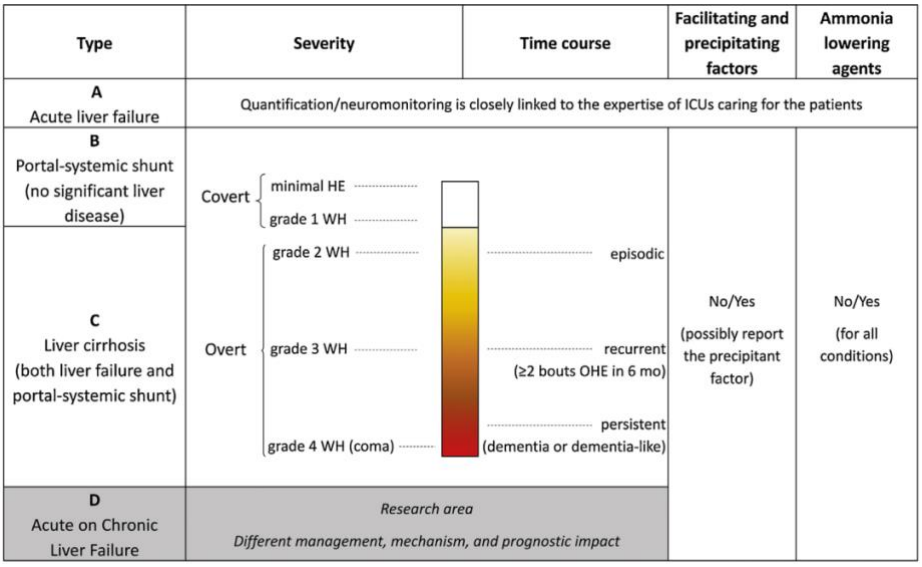


**Figure 1** - Classification of HE according to AASLD/EASL guidelines.

The underlying disease is the first aspect to consider and classifies HE into three categories (i.e., type A, B and C).

Type A HE develops in the context of acute liver failure and is characterized by a rapid change in mental status, and may be accompanied by brain edema. Type B HE is often unsuspected as it is

caused by portal-systemic shunt even without significant liver disease and, as a consequence, standard liver tests can be normal. Lastly, type C HE is the most common form and occurs in patients with chronic liver disease (4).

The second parameter used to classify HE is disease severity, which is commonly assessed using the West Haven criteria. This grading system classifies HE into five stages, from grade 0 to grade 4, according to the severity of neurological and cognitive impairment. Grade 0 indicates the absence of clinically detectable abnormalities, whereas grades 1 through 4 reflect progressively worsening of neurological impairment, ranging from cognitive changes to coma (3). Although the West Haven criteria remain the most widely used tool for grading HE severity, it became evident that some patients may exhibit cognitive deficits despite having no obvious abnormalities on routine clinical examination. These subtle impairments can only be detected through specialized neuropsychological testing. To better capture this spectrum of disease, HE has also been categorized into covert HE (CHE) and overt HE (OHE), as shown in Figure 2.

CHE includes:

- minimal HE (MHE), in which patients have no symptoms and can be identified only through specific neurophysiological tests.
- Grade 1, which is characterized by subtle cognitive changes such as mild inattention, lack of awareness, and sleep disturbances.

OHE includes grade 2, 3 and 4 of the West Heaven criteria and is clinically characterized by spatial or temporal disorientation, asterixis and, in advanced stages, stupor and coma (3).

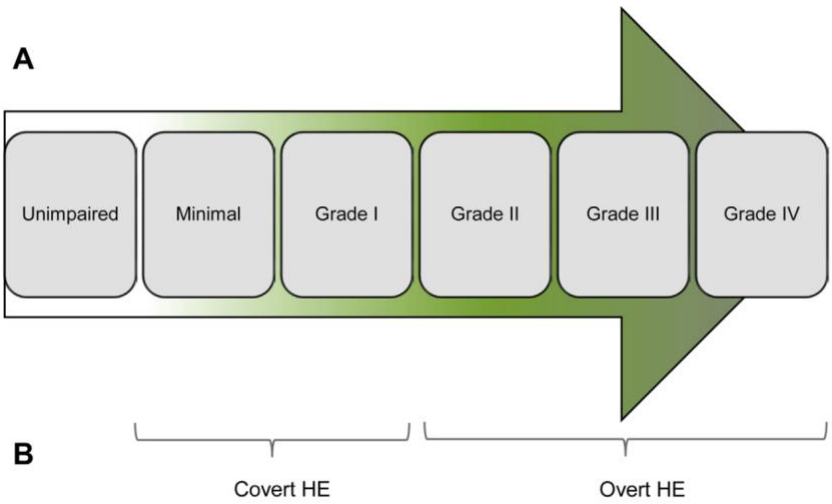


**Figure 2** - Classification of HE into covert and overt forms.

Further, HE can be classified as episodic, when it occurs at intervals greater than six months; recurrent, which is defined as more than two episodes within six months; persistent, when neuropsychiatric symptoms are stable and do not resolve between episodes.

Lastly, in relation to the presence of precipitating factors this syndrome is divided into spontaneous and precipitated HE. Common precipitating factors include: infections, constipation, gastrointestinal bleeding, dehydration, electrolyte disturbances, and renal dysfunction (3).

### 1.3 Incidence, prognostic impact, costs and social consequences

30-45% of patients with cirrhosis develop OHE over the course of the liver disease and this percentage becomes even higher in patients undergoing transjugular intrahepatic portosystemic shunt placement (TIPS), with a prevalence rate of 50% (5). CHE is more common, even though is frequently underdiagnosed due to the absence of clear symptoms; with neuropsychological/neurophysiological tests, it can be detected in up to 80% of patients with cirrhosis (6).

The development of a first episode of OHE is associated with a poor prognosis, with a survival probability of 42% at one year that decreases to 23% at three years (7).

The impact of HE extends well beyond direct medical consequences and encompasses important indirect challenges for patients, such as the

reduction of working ability and productivity. The unemployment rate of patients with hepatic encephalopathy is 87.5% compared to only 19% in patients who only suffer from cirrhosis (8). Quality of life is also impaired with heavy impact on social functions, sleep-wake cycle, and caregivers burden. HE affects the entire family, and often relatives end up handling daily care, causing both emotional and financial stress (9) and the development of anxiety or depression in nearly 25% of caregivers, who may experience difficulty maintaining their working position, leading to even higher indirect costs (4).

### 1.4 Pathophysiology

Hyperammonemia represents a key mechanism in the pathogenesis of the disease (Figure 3). This compound is produced both in the gastrointestinal tract by bacterial urease, and during amino acid catabolism. In the physiological state, the liver removes ammonia through the urea cycle, producing urea that is then eliminated by renal filtration. Extrahepatic organs, in particular skeletal muscle, also contribute to ammonia homeostasis. Under resting conditions, this tissue detoxifies ammonia through glutamine synthetase reaction, thereby compensating for impaired hepatic elimination in cirrhotic patients (10).

In the presence of chronic liver disease (e.g. cirrhosis) or portosystemic shunt, ammonia bypasses the hepatic metabolism and enters the systemic circulation (11). Once in the bloodstream, ammonia crosses the blood-brain barrier and enters astrocytes, where it is converted into glutamine by the glutamine synthetase enzyme. Glutamine creates an osmotic gradient that draws water into the glial cells, leading to a specific pattern of low-grade swelling named Alzheimer type II astrocytosis (4). Astrocyte swelling contributes to low-grade cerebral edema and impairs the normal regulatory functions of these cells. In addition, the accumulation of glutamine within astrocytes can induce mitochondrial dysfunction and increase the production of reactive oxygen and nitrogen species. These alterations disrupt neuron-astrocyte communication and impair neurotransmission (12).

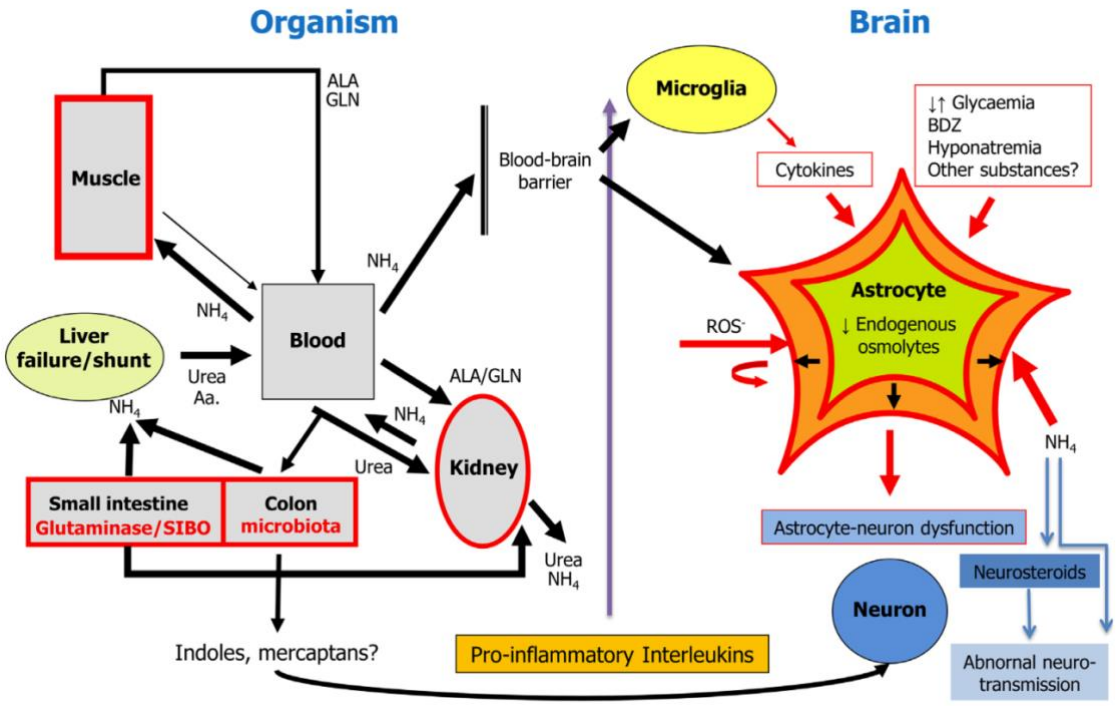


**Figure 3** - Pathophysiology of HE, highlighting the central role of ammonia.

Systemic inflammation also plays an important role in the development of HE. Pro-inflammatory cytokines, such as TNF-alfa, IL-1, and IL-6, increase the blood-brain barrier permeability, thereby facilitating the uptake of ammonia by astrocytes. In addition this molecules amplify the neuroinflammatory response within the brain by activating resident microglial cells (13).

Another aspect in the pathogenesis of hepatic encephalopathy is the gut-brain axis. Alterations in the gut microbiota have been observed in both covert and overt forms, with an increase in Enterobacteriaceae and Streptococcaceae and a reduction in Lachnospiraceae, Ruminococcaceae, and Clostridiales XIV. This dysbiosis has been associated with increased ammonia production, higher levels of endotoxins, and systemic inflammation (14).

### 1.5 Precipitating factors and comorbidities

OHE episodes are triggered by identifiable precipitating events, that work by increasing the nitrogen load, activating the inflammatory response, or altering cerebral osmolality. These factors have been grouped into five main categories. Infections, such as spontaneous bacterial peritonitis, urinary tract infections, and pneumonia, represent the most frequent trigger, identified in 43-49% of the cases. Acute variceal bleeding and constipation are others major precipitants that lead to a rapid rise in ammonia production. Finally, dehydration, excessive use of diuretics, and electrolyte disturbances, particularly hyponatraemia, can contribute to the development of hyperammonemia (2). The prompt correction of these factors is a key part of the therapeutic plan. When cognitive dysfunction persists, despite the clinical management of these features, clinicians should consider other causes of altered mental status (4).

Several comorbidities contribute to HE susceptibility: advanced age, for example, is a well-known risk factor because the elderly population has a reduced neurocognitive reserve (2). Benzodiazepines and opioids are often considered “false precipitants” because they do not directly increase ammonia levels but can cause changes in mental status that resemble HE. However, benzodiazepines can increase the brain's sensitivity to ammonia, making hepatic encephalopathy more likely to occur even at lower ammonia levels (4). Lastly, as skeletal muscle is an important site for ammonia detoxification, alterations in body compositions (i.e. sarcopenia) also increase the risk of HE (2).

### 1.6 Clinical presentation of hepatic encephalopathy

Covert HE represents the early stages of the syndrome when symptoms are subtle and difficult to detect clinically. At this preclinical stage the most affected domains are: attention, vigilance, response inhibition, executive functions, visuomotor coordination, and working memory (15). In Grade 1 HE, symptoms are usually mild and include slight changes in attention, concentration, behavior, and sleep patterns. These changes may not be

obvious to others but can often be noticed by family members, caregivers, or primary care physicians (1).

Overt HE encompasses West Haven grade II, characterized by lethargy, apathy, disorientation for time, obvious personality changes, inappropriate behavior, dyspraxia, and asterixis. As the condition progresses, patients can experience somnolence, confusion, space disorientation, bizarre behavior up to semi-stupor, with retained responsiveness to external stimuli. The last stage of the disease is defined as grade IV when patients are in a comatose state, as illustrated in Figure 4 (16).

<table>
<tr><th>Clinical features</th><th>Clinical definition</th><th>Research definition</th></tr>
<tr><td>No specific symptoms and normal specialized testing results.</td><td>Unimpaired</td><td>Unimpaired</td></tr>
<tr><td>No specific symptoms but abnormal specialized testing results.</td><td rowspan="2">Covert</td><td>Minimal</td></tr>
<tr><td>Nonspecific changes without asterixis with abnormal specialized testing results.</td><td>Grade I</td></tr>
<tr><td rowspan="3">Disorientation, asterixis, change in consciousness through coma. Specialized testing not required for diagnosis.</td><td rowspan="3">Overt</td><td>Grade II</td></tr>
<tr><td>Grade III</td></tr>
<tr><td>Grade IV (coma)</td></tr>
</table>

**Figure 4** - Clinical presentation of covert and overt HE.

### 1.7 Diagnostic approach to hepatic encephalopathy

To identify CHE specialized psychometric tests are used, the simplest one being the animal naming test (ANT), that consists of listing as many names of animals as possible in one minute (17). The gold-standard exam for CHE is a battery of five paper-and-pencil tests known as Psychometric Hepatic Encephalopathy Score (PHES), that assess: psychomotor speed and precision, visual perception, visuo-spatial orientation, visual construction, concentration, attention, and memory (18). Performance on the Number Connection Tests A and B, the Serial Dotting Test, and the Line Tracing Test are measured in seconds, whereas the Digit Symbol Test is scored according to the number of correct symbols completed

within 90 seconds. The results of all tests are then converted into standardized scores (Z-scores) and combined into a total PHES score; a PHES score ≤ −4 is considered abnormal. The Z-scores interpretation should consider many confounding factors, including age, educational level, and comorbidities (i.e., dementia and Parkinson's disease) (18). The Mini-Mental State Examination (MMSE) is not routinely used for the diagnosis of HE, as it lacks sensitivity for detecting the subtle cognitive deficits characteristic of this condition. However, it may be useful in the differential diagnosis to identify other neurocognitive disorders, such as dementia, that can mimic or contribute to cognitive impairment in patients with HE (15). In addition to psychometric tests, electroencephalography (EEG) can be used to evaluate brain dysfunction in patients with CHE. EEG may reveal subtle abnormalities in cerebral activity and can help identify early stages of the disease (19).

The diagnostic approach to OHE varies according to the underlying liver disease. In patients with cirrhosis, the diagnosis is primarily based on clinical assessment. The first step is the recognition of neurological and psychiatric manifestations suggestive of OHE, including confusion, disorientation, personality changes, altered consciousness, and asterixis (20). After the clinical evaluation, fasting venous ammonia levels should be measured. Although elevated ammonia levels alone are not sufficient to establish the diagnosis, they support the clinical suspicion of OHE. Clinicians should also evaluate the presence of any precipitating factor (20). Comatose patients should be assessed using the Glasgow Coma Scale (GCS), which helps define the severity of neurological impairment and allows clinical monitoring over time (21).

The diagnostic work-up is different in patients with acute liver failure (ALF). In this setting, the evaluation should include serum aminotransferases, bilirubin, coagulation tests, and other laboratory parameters used to assess the severity of liver failure (22). The grade of encephalopathy should also be assessed as it has prognostic implications and may influence decisions regarding intensive care management and liver transplantation (21).

In patients with spontaneous or surgical portosystemic shunts, the diagnostic process includes the identification of neurological manifestations compatible with OHE along with radiological confirmation of the shunt. Imaging techniques such as contrast-enhanced computed tomography, magnetic resonance imaging, or Doppler ultrasound may be used (23).

When ammonia levels are normal, alternative causes of altered mental status should be investigated. Depending on the clinical context, the diagnostic work-up may include brain computed tomography to exclude intracranial hemorrhage or other structural brain lesions, serum sodium measurement to identify hyponatremia, renal function tests, blood glucose evaluation, infection screening, and assessment for alcohol withdrawal, drug toxicity, or other neurological disorders (20).

EEG can be used to assess brain dysfunction in patients with HE. Although EEG findings are not specific to HE, they can support the diagnosis and evaluate the severity of the disease (19). As HE progresses, EEG activity becomes progressively slower. Common findings include slowing of the background rhythm, reduced reactivity to eye opening, reduced alpha activity, and increased theta and delta activity (19). EEG can also be analyzed using quantitative techniques, such as spectral analysis. These methods can detect subtle abnormalities even in the early stages of the disease and can help distinguish different grades of HE. For example, an increase in theta-band activity above 35% has been associated with mild hepatic encephalopathy (24). Another useful parameter is the mean dominant frequency (MDF), which represents the average frequency of the dominant background EEG activity. As HE worsens, MDF progressively decreases (19). Because EEG abnormalities may also occur in other metabolic, toxic, or neurological conditions, EEG findings should always be interpreted with the patient's clinical presentation and other diagnostic tests (19).

### 1.8 Therapeutic management of hepatic encephalopathy

The management of an OHE episode generally requires the identification and correction, if any, of the precipitating factors, and a prompt initiation of ammonia-lowering therapy. Treatment of precipitating factors is essential to control the acute episode. Infections should be actively searched for and treated with appropriate empirical or targeted antibiotic therapy. Gastrointestinal bleeding requires stabilization and specific management, while dehydration should be corrected with intravenous fluids. Electrolyte imbalances (i.e., hypokalemia or hyponatremia) should be corrected, and sedative medications should be discontinued when possible. Constipation should be treated with laxatives to promote regular bowel movements and reduce intestinal ammonia production (1).

The most common ammonia-lowering drugs are the non-absorbable disaccharides, such as lactulose or lactitol, and non-absorbable antibiotics (i.e. rifaximin). Generally, the non-adsorbable disaccharides are used as secondary prophylaxis as a standalone therapy; after a second OHE episode, a non-absorbable disaccharide is added. Primary prophylaxis is not generally performed, except for the administration of lactulose to rapidly remove blood from the gastrointestinal tract after a gastrointestinal bleeding (25). Lactulose works by acidifying the colon to convert ammonia ($NH_3$) into non-absorbable ammonium ($NH_4^+$). Chronic use of lactulose has been associated with changes in the gut microbiota, including an increase in beneficial saccharolytic bacteria and a reduction in ammonia-producing bacterial activity (26). In addition, lactulose has a clear cathartic effect, increasing stool water content and accelerating intestinal transit. This reduces bacterial ammonia production by decreasing the time available for protein degradation in the colon (27). Rifaximin is often added to lactulose and acts by binding to the bacterial DNA-dependent RNA polymerase, thereby inhibiting bacterial RNA synthesis. The therapeutic goal is to achieve two or three soft bowel movements per day (16).

Branched-chain amino acids (BCAAs) are an additional therapeutic option for HE. In cirrhotic patients, plasma levels of BCAAs are often reduced,

while aromatic amino acids are increased, leading to altered neurotransmission in the brain. Supplementation with BCAAs may help restore this balance, improve nitrogen metabolism, and support ammonia detoxification in skeletal muscle (1).

Another pharmacological option is L-ornithine L-aspartate (LOLA), which reduces ammonia levels by enhancing its detoxification through the urea cycle in the liver and by promoting ammonia metabolism in skeletal muscle (25).

There is evidence that nutritional management is important in patients with HE. Current recommendations suggest an energy intake of about 35-40 kcal/kg/day and a protein intake of 1.2-1.5 g/kg/day to avoid malnutrition and muscle loss (1). Protein should not be restricted, but the source of protein can be modified. Vegetable and dairy proteins are preferred, while excessive intake of meat should be limited, as it may increase nitrogen production. Patients should also eat small and frequent meals during the day. Long periods of fasting should be avoided because they can increase muscle breakdown and ammonia production. A late-evening snack is also recommended to reduce fasting during the night (1).

When patients do not respond to medical therapies, interventional procedures may be considered. Embolization of large spontaneous portosystemic shunts (11) or reduction of transjugular intrahepatic portosystemic shunt diameter can redirect portal blood flow through the liver, thereby improving hepatic detoxification (28). Lastly, liver transplantation remains the only definitive cure for HE as it directly addresses the underlying liver failure (11).
Finally, the provision of basic information on HE pathophysiology and simple hygienic and behavioural norms has a significant impact on quality of life. Even a 15-minute counseling session has been shown to be highly effective in preventing new episodes of hepatic encephalopathy (25).

## 1.9 Electroencephalography in hepatic encephalopathy

### 1.9.1 The role of electroencephalography in HE diagnosis

EEG is a non-invasive technique that records the electrical activity of the brain through electrodes placed on the scalp. It reflects the summed activity of cortical neurons and is commonly used to evaluate brain function in different neurological and metabolic conditions, including HE (29). EEG activity is conventionally divided into distinct frequency bands, namely delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), and beta (13-30 Hz). In healthy awake adults, the dominant rhythm is the alpha activity, especially in the posterior regions of the brain.

EEG analysis can be performed in two ways: a qualitative analysis, based on visual interpretation of the recording by an experienced neurophysiologist; a quantitative analysis, which uses mathematical methods to measure frequency and amplitude changes in brain activity (29). In HE the most relevant neurophysiological change is a progressive slowing of background brain activity of the wake EEG. In early stages, the normal alpha rhythm becomes less organized and is increasingly mixed with theta activity. As the disease progresses, theta waves become more dominant (29). In more advanced stages, triphasic waves may be observed. These consist of two electronegative waves followed by a positive wave of higher amplitude. However, these patterns are neither specific or pathognomonic to HE, as they can be observed in other metabolic encephalopathies (30).

The relationship between EEG abnormalities and the patient's clinical status was first described by Parsons-Smith and coworkers, who suggested a classification (Figure 5), which separates EEG changes into five stages (0-4 or A-E) based on the main background frequency. At one end of the spectrum, grade A shows a normal alpha rhythm (8-12 Hz). Early changes appear in grade B, where the alpha rhythm becomes unstable and mixes with theta waves (5-7 Hz). In grade C, the theta activity becomes dominant, though some alpha rhythm may still be present. In more advanced stages, it appears grade D, which is defined by

mixed theta and delta activity (0.5-4 Hz). Finally, Grade E is the most severe, with mainly low-frequency delta activity (30). Davies et al. showed that EEG slowing increases with the severity of HE. This finding suggests that EEG abnormalities are closely related to the clinical phenotype of the disease (30).

| Type | Characteristics | Dominant Frequency [b] |
|---|---|---|
| A | Decreased alpha activity<br>Faster potentials in all leads.<br>Generally flat and featureless. | Alpha |
| B | Unstable alpha rhythm.<br>Random 5-7Hz signal. | Alpha & Theta |
| C | Alpha rhythm still present.<br>Runs of 5-6z signal. | Alpha & Theta |
| D | 5-6Hz signal in all cases. | Theta |
| E | 2Hz signal replaces 5-6Hz signal<br>and is dominant over frontal lobes | Delta |

**Figure 5** - The Parsons-Smith classification, which categorizes EEG changes in HE into five stages according to progressive slowing of the background activity.

Classical visual EEG interpretation has several limitations. A primary concern is the lack of specificity, as similar patterns can also appear in other metabolic encephalopathies (31). In addition, qualitative EEG is inherently subjective, leading to inconsistencies in grading. These limitations have led to the development of more objective quantitative analysis (32).

Despite these limitations, EEG continues to play an important complementary role in the clinical evaluation of HE, as it can reveal abnormalities even when there are no obvious clinical signs. It is particularly useful to detect covert HE (31). EEG is also valuable in the differential diagnosis of altered mental status and has prognostic value, since the degree of EEG abnormalities often correlates with disease severity (33).

### 1.9.2 The transition to quantitative EEG: principles ad advantages of spectral analysis

Quantitative EEG (qEEG) analysis is primarily based on mathematical algorithms: the most important is the fast Fourier transform (FFT), which decomposes the raw EEG signal into its constituent frequency components, allowing researchers to calculate several critical indices. Among these, the mean dominant frequency (MDF) is a global measure of the background rhythm, calculated by weighting each frequency component's power by its corresponding frequency rate (34). Moreover, the relative power distribution breaks down the EEG spectrum into the usual frequency bands: delta (1-3.5 Hz), theta (4-8 Hz), alpha (8.5-13 Hz), and beta (13.5-26.5 Hz), which are associated with distinct types of cerebral activity (31). Further, additional parameters, such as the occipital alpha/theta ratio (LogR), have been proven useful for accurately identifying low-grade cerebral dysfunction (34).

The adoption of quantitative EEG has introduced several advantages. Unlike psychometric tests, it can be performed in non-cooperative patients, it is almost completely independent on operator interpretation and is also highly repeatable: while visual readings can vary significantly between different operators, automated approaches demonstrate superior reading repeatability (32). Studies have also shown that qEEG can detect subclinical brain dysfunction in approximately 31% of cirrhotic patients, compared to only 23% identified through traditional visual EEG (34). The clinical relevance of qEEG is further supported by its documented association with markers of liver dysfunction (i.e., Child-Pugh score) and with neuropsychological tests (i.e., PHES) (34). Quantitative EEG parameters also carry prognostic information: lower MDF and increased relative theta power are independent predictors of 12- and 18-month mortality (35).

Despite its ability to provide an objective and quantitative assessment of EEG abnormalities, spectral analysis has some important limitations. It requires dedicated equipment and specific expertise for data acquisition and interpretation, which are not always available in routine clinical

practice (19). Moreover, quantitative EEG analysis is usually performed in specialized neurophysiology centers and is not commonly used by hepatologists or gastroenterologists. The technique may also increase costs and require additional time compared with standard clinical evaluation (19). Finally, although spectral EEG analysis is useful for assessing disease severity and monitoring patients over time, its ability to detect mild forms of HE remains controversial, reducing its value as a stand-alone diagnostic tool (19).

The model for end-stage liver disease (MELD) is widely used to assess liver disease severity and prioritize patients for liver transplantation. The standard MELD score is based on three laboratory parameters: serum bilirubin, international normalized ratio (INR), and serum creatinine (36). Since HE is not included in the standard MELD score, quantitative EEG has been added in the MELD-EEG model to include brain dysfunction in prognostic evaluation. This combined model may improve outcome prediction compared with MELD alone. However, its use in clinical practice is still limited because EEG requires specific equipment and expertise and is not routinely performed in hepatology (35). In addition, the model has not yet been validated in large independent studies, and EEG changes are not specific to HE (35).

### 1.9.3 Application of machine learning and artificial intelligence to EEG in HE

The clinical diagnostic landscape of HE is evolving, with artificial intelligence (AI) and machine learning (ML) being commonly used in clinical practice. In this setting, ML techniques are great at identifying subtle and complex patterns in neurophysiological signals that are not obvious in classical visual EEG readings, even for experienced clinicians.
The use of AI in HE has gone through several stages as technology has improved. At first, researchers used hybrid systems that combined artificial neural networks (ANNs) with rule-based expert systems. One well-known example is the ANNES model, which was designed to mimic how an EEG specialist makes decisions. This system showed that it could sort EEG recordings into the five clinical stages of HE with high accuracy (37).

Supervised learning methods, such as support vector machines (SVMs), have also been used in HE studies. SVMs are especially good at solving classification problems as they can project data into higher-dimensional spaces where distinctions between classes become more clearly defined (38).

Deep learning (DL) models are the latest step forward: they use multi-layer neural networks to automatically extract salient features from raw EEG signals, making DL promising for analyzing complex neurophysiological data (37).

Using machine learning for EEG analysis has several advantages over traditional EEG analysis. One of the main benefits is that ML-based methods provide objective and reproducible insights into brain dysfunction, which is useful for long-term follow-up and monitoring the disease progression (35). Another important advantage is that artificial neural network-based EEG analysis provides a standardized and objective assessment of EEG abnormalities associated with CHE, potentially improving the detection of subtle changes that may be missed by conventional visual grading (32). ML models can also help predict patients' prognosis. As more studies confirm their usefulness and they become more common in clinical practice, ML tools could make a big difference in optimizing candidate selection for liver transplantation and personalized therapies (37).

### 1.9.4 Limitations of EEG processing in Hepatic Encephalopathy and the need for automated segment selection

In the current clinical workflow, EEG analysis in HE is only partially automated. Although spectral analysis provides quantitative and objective measures of brain activity, it still requires an initial manual assessment. Clinicians are required to visually inspect a standard 10-minute EEG recording and select a one-minute artifact free-segment representative of the patient's wakeful state. This step is necessary because artefacts such as eye movements, muscle activity, or reduced alertness can significantly distort quantitative EEG measures and affect the reliability of the analysis (19).

This manual selection introduces subjectivity and inter-observer variability, as the choice of the appropriate EEG segment depends on the clinician's experience. In addition, many hepatologists and gastroenterologists are not specifically trained in EEG interpretation, which further limits its reproducibility and consistency in routine clinical practice (19).

As a consequence, although the subsequent spectral analysis can be semi-automated and standardized, the overall pipeline still relies on a human decision at a critical step. This represents a methodological bottleneck and a major limitation for large-scale application. These issues provide the rationale for developing ML-based approaches capable of identifying artifact-free EEG segments suitable for quantitative analysis and clinical interpretation.

## 2 AIM OF THE STUDY

The aim of this study is to develop a machine learning-based system designed to automatically select a one-minute noise-free wake EEG segment for subsequent spectral analysis, thus fully automating EEG analysis for purposes of HE diagnosis/grading.

# 3 MATERIALS AND METHODS

## 3.1 Outpatient evaluation

Between 2009 and 2026, patients with liver disease referred to the HE outpatient clinic of the Regional Centre for Liver Disease at Padova University Hospital, were evaluated for HE diagnosis, treatment optimization, and differential diagnosis of other causes of altered mental status (e.g. dementia). For each patient, a paper record was completed and included:

- Demographics, occupation, educational level, smoking habits, and alcohol consumption
- Medical history focused on liver disease and its complications (i.e., gastrointestinal bleeding, varices, ascites, and previous episodes of HE), the presence of TIPS, and other relevant comorbidities
- Detailed medication history, investigating the use of tranquilizers, major sedatives, antidepressants, antiepileptic drugs, and other ongoing treatments, including HE therapy
- Physical examination focused on assessing alertness, cooperation, motivation, mood, the presence of asterixis and tremor, and alterations of the sleep-wake cycle
- Recent blood tests including: haemoglobin, platelet count, fasting blood glucose, urea, creatinine, serum electrolytes, proteins and albumin, total and fractioned bilirubin, INR, venous fasting ammonia
- Recent abdomen ultrasound findings, to evaluate complications related to cirrhosis, including hepatomegaly, splenomegaly, hepatic lobe atrophy, and presence of spontaneous portosystemic shunts
- Recent upper gastrointestinal endoscopy findings, including the presence of hypertensive gastropathy, esophageal varices, and peptic ulcer disease.

The neuropsychological battery administered included: the MMSE, the Animal Naming Test (ANT), the Psychometric Hepatic Encephalopathy Score (PHES), and selected components of a brief neuropsychological battery called *“Esame neuropsicologico breve 2”*.

PHES battery includes: the Trail Making Test A and B, the Digital Symbol test, the Line Tracing test, and the Serial Dotting test. Both the mean z score (MPZS) and rounded sum of each test z score (PHES), adjusted for age and educational level in relation to Italian norms were obtained (18). A PHES score ≤ - 4 was considered abnormal.
The ANT requires the patient to list as many animals as possible in one minute: a result of < 15 animals was considered abnormal (17).
The Mini Mental State Examination (MMSE) consists of a series of questions assessing different cognitive domains, including orientation in space and time, attention, memory, language, visuospatial skills and executive functions. The test score ranges from 0 to 30, and cognitive impairment was considered when the score was ≤ 24.

Subsequently, patients underwent a 11-minute EEG recording, including 3 minutes with eyes open and 8 minutes with eyes closed.
EEG was recorded by 21 channels, with the ground placed on Fpz and the reference on Oz and included reference derivations, electrocardiographic monitoring, and ocular movement recording. Impedance was kept below 5kΩ and the signal was filtered in the range of 0.33-60 Hz. Sampling frequency was 256 Hz and conversation resolution 0.19 mV/digit. After selection of one minute artifact-free wake EEG section, spectral analysis was performed on P3-P4. The mean dominant frequency (MDF) and the relative amount of slow EEG activity within the theta and delta frequency bands were calculated. The EEG was considered abnormal if MDF was less than or equal to 7.3 Hz or if relative theta/delta power was greater than or equal to 35/44%, respectively.

Prior to 2009, evaluations of patients with altered mental status/HE were performed, but no structured database (paper record or a database) was recorded. As a result, only EEG recordings were systematically archived, while other clinical, laboratory, and psychometric data were not consistently collected in a standardized and retrievable format.

### 3.2 Database completion

A pre-existing database created in 2009 was updated by adding data from new evaluations between 20 January 2025 and 22 May 2026, as shown in Figure 6. Demographic data were collected, including sex, age, and educational level. A series of clinical data were also recorded, including etiology of cirrhosis, ascites, presence of HCC, presence of spontaneous/surgical/trans jugular portal-systemic shunt, asterixis, previous varices bleeding, diabetes, previous episodes of HE, MELD score, and Child Pugh score. Laboratory parameters were also collected, including venous blood ammonia, albumin, total bilirubin, INR, creatinine, TSH, and PCR. The results of the PHES test (i.e., the Trail Making Test A and B, the Digital Symbol test, the Line Tracing test, the Serial Dotting test, the MPZS, and the PHES score) and of the artifact-free wake EEG segment (i.e., MDF and theta and delta EEG power) were also collected. The database also recorded whether the patient was already on secondary prophylaxis for HE with rifaximin and lactulose. The degree of HE (i.e., grade 0, 1, 2 or 3) was assessed according to the diagnostic algorithm proposed by the AISF 2022 guidelines (1).

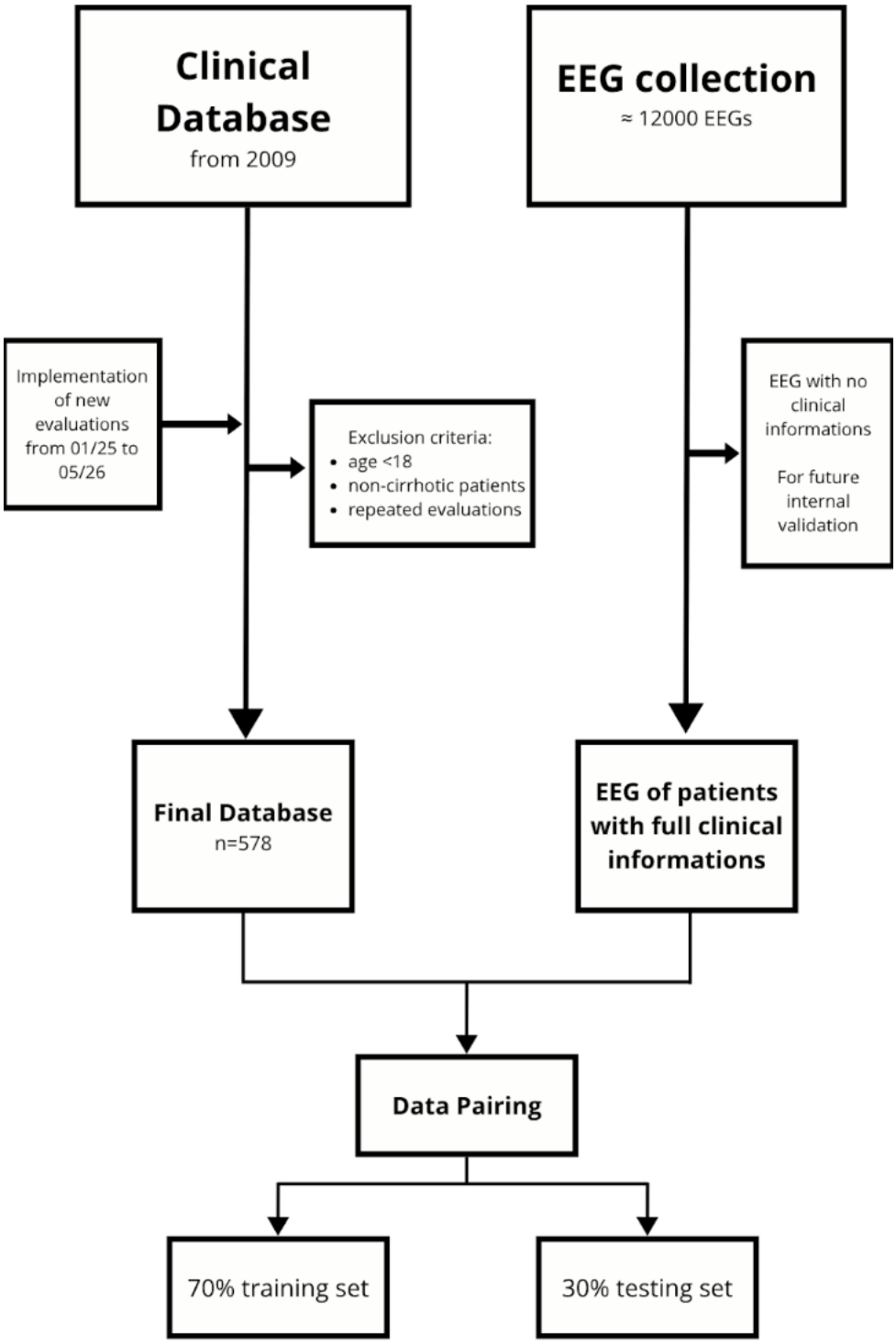


**Figure 6**. Schematic representation of the proposed EEG processing and machine learning pipeline for HE diagnosis/classification.

### 3.3 Definition of the problem and organization of the EEG database

A total of approximately 12,000 EEG recordings (about 110 GB of data) were retrieved from the digital archive of the Clinica Medica 5°, including all available recordings collected between 2009 and 2026. All files were stored in proprietary TRC format (Micromed), which contains both electrophysiological signals and embedded metadata such as patient identifiers and acquisition date. The entire dataset was transferred to dedicated computational workstations for subsequent processing.

In parallel, the database was prepared to identify the subset of evaluations/EEG recordings suitable for the AI training. The exclusion criteria were: age <18 years, absence of cirrhosis, and repeated evaluations of the same patient. The final dataset included, for each evaluation, date of assessment, spectral EEG parameters (mean dominant frequency and relative theta/delta power), and the corresponding HE grading based on spectral analysis.

Importantly, for each EEG included in the clinical database, a one-minute artifact-free wake segment had been previously selected by expert clinicians for spectral analysis. These manually selected segments represent the reference standard for signal quality and were used as labeled data for subsequent computational processing.

This procedure allowed the creation of a structured and clinically annotated EEG dataset, in which each recording was linked to validated spectral parameters and a corresponding high-quality reference segment.

### 3.4 Definition of the solutions for automated data management

To enable automated analysis of EEG recordings, an integrated data processing workflow based on Python and MATLAB was designed. Python was selected for data extraction and organization, while MATLAB was used for signal processing and quantitative analysis.

The key methodological step consisted in linking EEG recordings to the corresponding clinical entries in the database. This matching procedure was performed using information contained in both the TRC files (patient

identifiers and recording date) and in the database, allowing the identification of EEG traces associated with patients included in the study dataset and facilitating comparison with the previously performed spectral analysis available in the provided dataset.

The workflow was developed to extract key metadata from EEG recordings, including temporal information and clinical annotations, and to transfer these data into intermediate Excel files that could be subsequently imported into MATLAB. This approach was adopted to facilitate large-scale automated analysis while avoiding manual preprocessing steps.

As the recordings were collected over several years and annotated by different clinicians, identical events were often labeled using different terms (e.g., "eyes open", "open eyes", or "occhi aperti"). Given the heterogeneity of annotations, a semantic generalization strategy was implemented to identify different linguistic variants referring to the same clinical event. This approach enabled the automated recognition of equivalent annotations throughout the database.

Preliminary analyses were conducted to validate the signal processing pipeline and ensure consistency with the original spectral analysis. A small subset of 4 EEG recordings from the dataset was randomly selected, and for each recording the same one-minute artifact-free segment previously identified by expert clinicians was extracted. Spectral analysis was then performed on these segments using the implemented algorithm, allowing verification of both the correct interpretation of temporal markers and the reproducibility of spectral EEG parameters.

The initial machine learning approach was based on supervised learning.

Several approaches for artifact management were considered during model design. An initial strategy involving the inclusion of artifact-contaminated EEG segments was evaluated but not adopted because of the high variability and complexity of EEG artifacts. Artifacts can arise from different sources, such as eye movements, muscle activity, or other physiological or external interferences, and they may appear differently across EEG recordings. For example, eye movement artifacts can vary depending on the direction of gaze, while muscle artifacts depend on the type and intensity of muscle activity and do not affect all channels in the

same way. This variability makes artifacts difficult to describe in a consistent way and limits the ability of machine learning models to reliably detect and interpret them. Consequently, the final training dataset was restricted to high-quality artifact-free EEG segments manually identified by expert electroencephalographers during clinical review.

### 3.5 EEG signal optimization and development of the machine learning models

An automated strategy for EEG segment selection was developed. EEG recordings were divided into consecutive 2-second epochs, and each epoch was independently evaluated according to predefined signal quality criteria. Epochs classified as contaminated by artifacts were excluded, whereas acceptable epochs were retained for quantitative analysis.

For the retained EEG segments, the Mean Dominant Frequency (MDF), a quantitative biomarker associated with hepatic encephalopathy, was calculated automatically.

To assess the performance of the automated pipeline, a validation procedure was planned in which EEG segments selected by the algorithm were compared with those manually identified by experienced clinicians. Manual annotations of suitable EEG segments were used as the reference standard.

The machine learning framework was designed to integrate quantitative EEG features with additional clinical and laboratory variables available in the database, including age, smoking habits, and blood test results. Feature selection procedures were planned to identify the most informative variables for classification.

Several machine learning algorithms were selected for evaluation, including Random Forest, Support Vector Machine (SVM), AdaBoost, and Generalized Linear Models (GLM). Future model optimization will address missing data management, class imbalance, and overfitting reduction.

# 4 RESULTS

## 4.1 EEG database construction and characterization

A total of 959 clinical evaluations were extracted from the hospital database, each including clinical information, laboratory data, psychometric tests, and associated EEG recordings when available. These evaluations were collected between June 2009 and May 2026. From these, 578 evaluations met the inclusion criteria and were included in the final study cohort (Figure 6).

In parallel, approximately 12,000 EEG recordings were retrieved from the hospital neurophysiology archive for screening. These recordings were first linked to the clinical database in order to identify complete clinical-EEG pairs. During this process, EEG recordings were divided into two groups:

1. EEGs with complete clinical information and successful matching to the clinical database
2. EEGs not in the clinical database. This group was not used for AI model development and will be used in the future for a second internal validation process.

After matching and screening, the final study database consisted of 578 clinical-EEG paired evaluations with confirmed diagnoses. This dataset was randomly divided into a training set (70%) for model development and a test set (30%) for internal validation (Figure 6).

All EEG recordings were stored in Micromed TRC format, which includes raw EEG signals together with clinical annotations collected during routine clinical practice.

Overall, this process resulted in a structured multimodal dataset of patients with cirrhosis and HE, integrating clinical, laboratory, psychometric, and EEG data for machine learning analysis.

## 4.2 Clinical and neurophysiological characteristics of the study population

Regarding the study population (n=578), the mean age of the patients was 59 ± 10.2 years and 71% were male. The most frequent etiologies of liver disease were alcohol-related cirrhosis (33%) and other causes (e.g. mixed aetiologies, 33%).

MELD and MELD-Na scores were 14.6 ± 5.8 and 16.7 ± 6.3.

44% of patients had no evidence of HE, 48% had CHE while 6% had OHE grade II and 2% had OHE grade III. Current treatment for HE was reported in 73% of evaluations.

Quantitative EEG spectral analysis was available in most examinations. Details of neurophysiological and neuropsychological characteristics are reported in Table I.

| | | **All evaluations (n=578)** |
|---|---|---|
| | Age (mean±SD, years) | 59 ± 10.2 |
| | Sex [n(%), males] | 411 (71%) |
| | Education (mean±SD, years) | 10 ± 4.0 |
| **Aetiology** | Alcohol [n(%)] | 189 (33) |
| | Viral [n(%)] | 165 (29) |
| | Metabolic [n(%)] | 31 (5) |
| | Other [n(%)] | 172 (33) |
| | Pugh (mean±SD) | 8.1 ± 2.1 |
| | Child (%, A/B/C) | 25/49/26 |
| | MELD score (mean±SD) | 14.6 ± 5.8 |
| | MELD-Na score (mean±SD) | 16.7 ± 6.3 |
| | Ascites (%, absent/mild/severe or refractory) | 54/30/16 |
| **HE** | No HE [n (%)] | 194 (44) |
| | Covert HE [n (%)] | 214 (48) |
| | Overt HE - Grade II [n (%)] | 25 (6) |
| | Overt HE - Grade III [n (%)] | 10 (2) |
| | Asterixis (%, absent/rare/frequent) | 81/12/7 |
| | Portal systemic shunt (%, absent/spontaneous/surgical) | 46/48/6 |
| | Current HE treatment [n(%), yes] | 388 (73) |
| | Ammonia [micromol/L, (mean±SD)] | 66.9 ± 44.2 |
| | Animal naming test (mean ± SD) | 13 ± 5 |
| | MPZS (mean±SD) | -0.88 ± 1.47 |
| **EEG spectral analysis** | MDF (Hz, mean ± SD) | 9.29 ± 2.22 |
| | δ (mean±SD) | 11.66 ± 12.23 |
| | θ (mean±SD) | 34.27 ± 18.57 |
| | α (mean±SD) | 36.01 ± 18.17 |
| | β (mean±SD) | 18.01 ± 12.16 |

**Table I**. Demographic, clinical, laboratory, psychometric, and EEG characteristics of the 578 evaluations included in the study.

### 4.3 Identification of technical limitations for automated EEG analysis

Preliminary inspection of the EEG archive highlighted important technical challenges that prevented immediate implementation of automated signal analysis.

Although EEG recordings could be imported into MATLAB through available Micromed plugins and the EEGLAB toolbox, reliable extraction of temporal metadata was not possible. In particular, the initial recording time (T0) could not be consistently retrieved, and annotations recorded during EEG acquisition were not recognized as structured events synchronized with the signal.

Furthermore, the clinical annotations contained in the recordings had been generated by different operators over several years and lacked standardization. The same event was frequently described using different terms and languages, making automated identification of clinically relevant EEG segments difficult.

These limitations represented a major obstacle to the development of a fully automated pipeline for large-scale EEG analysis.

### 4.4 Development of automated solutions for EEG processing and AI training

To overcome the limitations identified during preliminary analyses, an integrated Python-MATLAB workflow was developed.

Python-based procedures enabled direct extraction of metadata from TRC files, including recording start times, event markers, and temporal coordinates of annotations. The extracted information was automatically organized into intermediate files and subsequently imported into MATLAB for quantitative signal analysis.

The developed workflow eliminated the need for manual conversion of recordings into EDF format, a procedure that would otherwise have required file-by-file processing of the entire EEG archive.

To address annotation heterogeneity, semantic generalization procedures were implemented, allowing automatic recognition of different linguistic

variants describing the same clinical event (e.g., "occhi chiusi", "eyes closed", or "closed eyes").

In parallel, the material used for machine learning development was defined. A supervised learning approach was selected, using a training dataset consisting of approximately 70% of the total cohort of patients with established diagnoses and EEG recordings previously reviewed by expert clinicians (n=578).

An initial strategy based on explicit training of artifact recognition was evaluated but later abandoned because of the large variability of EEG artifacts and the difficulty of generating a comprehensive set of artifact examples. Consequently, the final approach focused on training the algorithm using artifact-free EEG segments selected by experienced electroencephalographers.

### 4.5 Definition of automated EEG segment selection and export procedures

A major objective of the project was the development of an automated procedure capable of identifying EEG segments suitable for spectral analysis without operator intervention.

In routine clinical practice, quantitative EEG spectral analysis is generally performed on approximately one minute of artifact-free wake EEG manually selected by expert electroencephalographers. In order to reproduce this procedure automatically, preliminary validation of the spectral analysis pipeline was first required. Therefore, an initial feasibility analysis was conducted on a limited subset of EEG recordings (n=4). For each recording, spectral analysis was reproduced in MATLAB using the same one-minute EEG segment previously selected by expert clinicians during routine clinical evaluation. The spectral parameters obtained through the implemented algorithm were then compared with those derived from the original manual analysis. The resulting values showed good agreement, supporting the reproducibility of the computational pipeline and allowing further development of the automated processing strategy (Table II).

Subsequent analyses focused on the identification of methods capable of automatically selecting 60-100 seconds of analyzable EEG activity, either as a single continuous artifact-free segment or as a combination of multiple shorter acceptable segments. Preliminary inspection of the recordings suggested that completely artifact-free one-minute intervals may be difficult to identify consistently in routine clinical EEGs. Therefore, an alternative strategy based on short-duration epochs was proposed. According to this planned approach, EEG recordings will be automatically divided into consecutive 2-second windows, and each epoch will be independently evaluated according to predefined signal quality criteria. Epochs classified as contaminated by artifacts will be excluded, whereas acceptable epochs will be retained for quantitative analysis.

Implementation of this strategy required the development of reliable procedures for extraction of temporal markers and event annotations from the original TRC files. The newly developed extraction workflow successfully provided the temporal references necessary to reconstruct the EEG timeline and to associate annotations with specific signal segments, thus creating the technical basis for future automated identification of candidate epochs for spectral analysis.

At the current stage of the project, automated epoch selection and large-scale comparison with manual EEG review have not yet been fully implemented. However, the preliminary organization of the database and the successful extraction of temporal and annotation metadata support the feasibility of subsequent development of automated spectral EEG analysis in large clinical datasets.

Now that the complete clinically annotated dataset has been defined, the proposed methodology will be applied and systematically evaluated on a larger scale in subsequent phases of the project.

| | | Participant 1 | Participant 2 | Participant 3 | Participant 4 |
|---|---|---|---|---|---|
| **Age (years)** | | 72 | 60 | 57 | 61 |
| **Sex** | | Female | Male | Female | Female |
| **Education (years)** | | 8 | 8 | 18 | 16 |
| **Aetiology** | | Metabolic | Metabolic | Metabolic | Mixed |
| **MELD score** | | 15 | NA | 17 | 29 |
| **MELD-Na score** | | 15 | NA | 18 | 29 |
| **Ascites** | | Absent | Absent | Absent | Mild |
| **HE grade** | | No HE | Covert | No HE | No HE |
| **Asterixis** | | Absent | Absent | Absent | Absent |
| **Portal systemic shunt** | | Present | Present | Absent | Absent |
| **Current HE treatment** | | Present | Present | Absent | Present |
| **Ammonia (micromol/L)** | | 15 | NA | 86 | 43 |
| **Animal naming test** | | 4 | 8 | 21 | 19 |
| **MPZS** | | -0.09 | -1.22 | 0.2 | -0.48 |
| **Manual spectral analysis P3-P4** | **MDF (Hz)** | 10.05 | 7.99 | 12.53 | 10.33 |
| | **δ (%)** | 2.84 | 6.99 | 2.21 | 7.45 |
| | **θ (%)** | 33.43 | 61.48 | 11.76 | 25.25 |
| | **α (%)** | 44.69 | 24.27 | 49.52 | 45.95 |
| | **β (%)** | 19.04 | 7.25 | 36.51 | 21.34 |
| **Algorithm spectral analysis P3-P4** | **MDF (Hz)** | 10.05 | 8.07 | 12.54 | 10.34 |
| | **δ (%)** | 3.09 | 7.49 | 2.22 | 7.28 |
| | **θ (%)** | 33.54 | 59.45 | 11.61 | 25.03 |
| | **α (%)** | 44.19 | 25.38 | 49.00 | 45.92 |
| | **β (%)** | 19.18 | 7.67 | 37.18 | 21.77 |
| **Manual-Algorithm relative difference (%)** | **MDF** | 0.0 | -1.0 | -0.1 | -0.1 |
| | **δ** | -8.8 | -7.2 | -0.5 | 2.3 |
| | **θ** | -0.3 | 3.3 | 1.3 | 0.9 |
| | **α** | 1.1 | -4.6 | 1.1 | 0.1 |
| | **β** | -0.7 | -5.8 | -1.8 | -2.0 |

**Table II –** Demographic, clinical, laboratory, psychometric, and EEG characteristics of a subgroup of 4 participants' evaluations comparing the manual and algorithm spectral analysis parameters.

## 5 DISCUSSION

The present study aimed to create a database integrating clinical and electroencephalographic (EEG) data as a foundation for the future development and implementation of machine learning algorithms. The collected dataset includes EEG recordings together with relevant clinical, neurophysiological, psychometric, and laboratory information, providing a comprehensive resource for subsequent data-driven analyses.

During the early phase of the project, several technical problems were identified. Standard EEG analysis software was not able to reliably extract timing information and structured annotations from proprietary TRC files. A combined Python-MATLAB workflow was developed, allowing direct extraction of metadata and automated processing of EEG recordings without manual conversion steps. In addition, clinical annotations collected over many years were highly variable. A semantic generalization approach was therefore used to group different expressions referring to the same events into common categories. Therefore, a substantial part of the project involved data harmonization and infrastructure development before ML applications could be implemented.

Finally, a method based on consecutive 2-second EEG windows was developed to identify artifact-free segments suitable for spectral analysis which has been shown to be preferable to wider windows (39). In preliminary testing, the automatically selected segments showed good agreement with those chosen by expert operators. Although evaluated on a limited number of recordings, this preliminary agreement suggests that automated EEG segment selection may reproduce the decision-making process of expert readers and therefore represents a promising step toward reducing operator-dependent variability.

These findings are particularly relevant in the context of HE, where progressive alterations in brain function are reflected by characteristic EEG slowing. Quantitative EEG parameters, including Mean Dominant Frequency and spectral power distribution, are widely used to evaluate these changes. However, their reliable calculation requires artifact-free

EEG segments, which are traditionally selected manually by expert readers. The automated approach developed in this study was designed to address this limitation and facilitate the use of quantitative EEG analysis in large clinical datasets. Together with the large multimodal database created during the project, it provides a comprehensive resource for future ML applications in HE, especially given that many previous EEG studies were based on smaller and more selected patient groups (19).

Quantitative EEG is a useful tool to detect brain dysfunction in HE. The main change seen in HE is a slowing of background activity, with lower mean dominant frequency and more slow-wave activity (19). Bahn et al. showed that these EEG changes can be used to follow disease progression in portal-systemic encephalopathy (40). However, these studies required experts to first manually select artifact-free EEG segments before analysis (40). For this reason, most research has focused on improving EEG features rather than automating data preprocessing (41). This distinction is important because segment selection represents a fundamental but often overlooked step in the EEG analysis pipeline. While many previous studies have concentrated on developing algorithms for HE diagnosis, staging, or outcome prediction, they generally relied on EEG data that had already been manually reviewed and selected by experts. By targeting the preprocessing stage, the present work aims to reduce operator-dependent variability and improve the scalability of EEG-based analyses. The epoch-based approach used in this study is similar to methods commonly adopted in EEG preprocessing. EEG recordings are often divided into short epochs that are individually evaluated for signal quality and the presence of artifacts before further analysis (42). In our study, artifact-free 2-second epochs were selected and then combined to obtain approximately one minute of EEG for spectral analysis, reproducing the procedure usually performed manually by expert readers.

Artificial intelligence has been applied to HE for many years, mainly to improve diagnosis and reduce inter-observer variability (32). A well-known example is ANNES, which was designed to mimic expert EEG

interpretation and improve HE staging consistency (32). However, it still relied on EEG data that had already been manually selected by experts (32). Recent ML methods have been used to detect and remove EEG artifacts, which has gained growing interest in EEG analysis (43). Some approaches use GAN-based models or other neural networks to reconstruct EEG signals affected by eye or muscle artifacts (43). In this study, the approach is different because it focuses on selecting naturally clean EEG segments instead of reconstructing the signal. This preserves the original EEG signal and more closely reflects routine clinical practice, where experts choose the best-quality EEG segments for analysis.

A main strength of this study is the large dataset collected over 17 years in a tertiary referral center. The inclusion of routine clinical EEG recordings, together with clinical, laboratory, and psychometric data, increases the potential applicability of the proposed approach in clinical practice. Another strength is the development of a workflow that directly extracts data from TRC files without format conversion. The use of semantic generalization also helped to standardize older clinical annotations and made the data easier to analyse.

Some limitations should be considered when interpreting this study. First, the automated EEG selection method was tested only on a small number of recordings, so the results are still preliminary. Second, all data come from a single center, so external validation is needed to confirm whether the method works in other settings. Third, clinical, laboratory, and psychometric data were not consistently available for all evaluations. This incomplete availability of clinical information may limit the development and validation of future multimodal machine learning models integrating EEG with clinical variables. Finally, this study focused on EEG segment selection and did not evaluate the performance of a final HE classification model.

Artifact-free EEG segments remain essential for reliable quantitative EEG analysis. Automating their selection could reduce operator-dependent variability, improve reproducibility, and facilitate the implementation of

quantitative EEG in large clinical datasets, multicentre studies, and routine hepatology practice (19). Standardized preprocessing may also support the adoption of EEG-based biomarkers and the use of quantitative EEG as an objective measure of disease progression and treatment response in HE (19). The next step of this work will be to test the automated selection method on the complete EEG archive and across different stages of HE. The large database also provides an opportunity for the future development of ML models for HE classification using quantitative EEG features. In the future, EEG-based variables could also be combined with clinical and laboratory data to improve prognosis in patients with HE.

## 6 CONCLUSION

In conclusion, this work suggests that ML can help automate the selection of high-quality EEG segments and may support more practical and reproducible EEG analysis in liver disease.

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