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Characterization and Evaluation of Alterations in Sensitivity and Autonomic Function in Cirrhotic Patients with Minimal Hepatic Encephalopathy

Dalia Angela Rega Caballero

Hospital Clínic Universitari de València

VNIVERSITAT DE VALÈNCIA

INCLIVA | VLC
Instituto de Investigación Sanitaria

Directors:
Dra. Carmina Montoliu Felix
Dra. Paula Cases Bergon

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To whom it may concern,

The doctoral thesis developed by me, Dalia Angela Rega Caballero, titled:

"Characterization and Evaluation of Alterations in Sensitivity and Autonomic Function in Patients with Minimal Hepatic Encephalopathy", includes data and results from the following publication of which I am the first author:

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I declare that this publication will not be used in any other thesis.

Sincerely,

Dalia Angela Rega Caballero

Dra. Carmina Montoliu Félix, investigadora de la Fundación para la Investigación del Hospital Clínico de la Comunidad Valenciana INCLIVA y profesora del departamento de Patología de la Universidad de Valencia.

Dra. Paula Cases Bergon, jefa del Departamento de Neurofisiología del Hospital Clínico de Valencia.

CERTIFICAN:

Que la memoria de Tesis Doctoral realizada por Dª Dalia Angela Rega Caballero, titulada "**Characterization and Evaluation of Alterations in Sensitivity and Autonomic Function in Cirrhotic Patients with Minimal Hepatic Encephalopathy**", ha sido realizada bajo su dirección y reúne todos los requisitos necesarios para su juicio y calificación.

En Valencia, 23 de octubre 2022

Carmina Montoliu Paula Cases

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Contents

Contents

Índice de abreviaturas

CDT *Cooling Detection Threshold* (Umbral de detección de frío)

ECN Estudio de Conducción Nerviosa

EHM Encefalopatía Hepática Mínima

HPDT *Heat Pain Detection Threshold* (Umbral de detección de calor por dolor)

IL Interleucina

JND *Just Noticeable Difference* (Umbral de detección)

PHES *Phychometric Hepatic Encephalopathy Score*

NEHM Sin Encefalopatía Hepática Mínima

PNP Polineuropatía

QST *Quantitative Sensory Testing* (Test de cuantificación sensorial)

ROC *Receiver Operating Characteristic* (Característica Operativa del Receptor)

RSC Respuesta Simpática Cutánea

SNP Sistema Nervioso Periférico

VDT *Vibration Detection Threshold* (Umbral de detección de vibración)

VFC Variabilidad de la Frecuencia Cardiaca

I Resumen

I. Resumen

Los pacientes con cirrosis hepática pueden experimentar alteraciones en el sistema nervioso periférico (SNP) y en la percepción somatosensorial. La alteración del sistema somatosensorial podría contribuir a los déficits cognitivos y motores, característicos en la encefalopatía hepática mínima (EHM), la cual afecta hasta a un 40% de los pacientes cirróticos. Se valoró la relación entre las alteraciones de sensibilidad térmica, vibracional y dolor en 58 pacientes cirróticos (38 sin EHM, 20 con EHM diagnosticada con la batería psicométrica, *Psychometric Hepatic Encephalopathy Score*, PHES) y 39 controles. Todos los participantes se sometieron a test neuropsicométricos, electromiografía y cuantificación sensorial para explorar la atención y coordinación, la conducción nerviosa periférica y autónoma, y los umbrales sensitivos (en las modalidades de vibración, frío y detección de dolor por calor). Los umbrales de detección de frío y calor-dolor en el pie, fueron mayores en los pacientes con EHM en comparación con los pacientes sin EHM. Esta hiposensibilidad se correlacionaba con los déficits de atención encontrados. Los tiempos de reacción en el pie fueron más largos en pacientes con EHM que en pacientes sin EHM. Los pacientes cirróticos con EHM con una amplitud normal del nervio sural, mostraron sensibilidad térmica alterada de forma significativa en comparación con pacientes cirróticos sin EHM. Los pacientes con EHM muestran un declive general en habilidades cognitivas y sensoriales. Las fibras finas del sistema nervioso autónomo y de sensibilidad térmica están alteradas de forma temprana en pacientes cirróticos con EHM, antes que las fibras gruesas. Los tests de cuantificación sensorial podrían ser una herramienta complementaria para detectar EHM de forma temprana.

1.1 Hipótesis y Objetivos

El deterioro cognitivo leve ha sido asociado a cambios tempranos en la corteza somatosensorial primaria, el cual ha sido propuesto como un marcador sensible para el deterioro cognitivo. Además de EHM, provocado por alteraciones de sistema nervioso central, es posible que los pacientes cirróticos puedan presentar alteraciones en el SNP. El estudio de alteraciones neurológicas, percepción sensorial, conducción nerviosa y función autónoma podría detectar EHM en fases más tempranas y con mayor sensibilidad que los métodos actuales utilizados para la detección de EHM.

El objetivo de este estudio fue evaluar y caracterizar la alteración de la sensibilidad térmica y mecánica y la velocidad de conducción nerviosa periférica en pacientes cirróticos con y sin EHM, su correlación con las alteraciones neurológicas y la posible contribución de la inflamación. Para abordar este objetivo general se desarrollarán los siguientes objetivos específicos:

1. Evaluación de distintas funciones neurológicas como la atención, la concentración, la velocidad de procesamiento mental, la memoria de trabajo y la coordinación bimanual y visomotora, mediante pruebas psicométricas específicas. Estas funciones se alteran en pacientes con EHM de forma temprana.
2. Evaluación de funciones motoras como el equilibrio, la fuerza de agarre y la velocidad motora, procesos que están íntimamente relacionados con el deterioro cognitivo y funcional.

3. Evaluación y caracterización de las alteraciones en la sensibilidad térmica, vibratoria y al dolor por calor en pacientes cirróticos y controles sanos, y valorar las diferencias entre pacientes cirróticos con y sin EHM.
4. Realizar estudios de conducción nerviosa sensitiva y motora, y pruebas de función autónoma para clasificar el tipo de fibra afectada (fibras finas o gruesas, sensoriales, motoras o ambas) e identificar el proceso patológico primario.
5. Correlacionar los resultados de rendimiento en alteraciones neuropsicológicas con alteraciones en la percepción sensorial y la función autónoma.
6. Evaluar la contribución de los parámetros inflamatorios a la alteración de la sensibilidad térmica, vibratoria y al dolor por calor asociada a la EHM.
7. Valorar la capacidad predictiva de alteraciones en la sensibilidad térmica y mecánica para la detección temprana de EHM.

1.2 Métodos

Cincuenta y ocho pacientes con cirrosis hepática fueron reclutados en las consultas externas de los hospitales Clínico y Arnau de Vilanova en Valencia, España. El diagnóstico de la cirrosis se basó en datos bioquímicos, clínicos y ultrasonográficos. Los criterios de exclusión fueron encefalopatía hepática clínica, ingesta reciente de alcohol (<6 meses), infección, uso de antibióticos, hemorragia gastrointestinal (<6 semanas), toma de drogas que afectan la función cognitiva (<6 semanas), presencia de carcinoma hepatocelular y/o trastornos neurológicos y psiquiátricos. Los pacientes diabéticos insulinodependientes también fueron excluidos ya que presentaban polineuropatías más severas que aquellos pacientes diabéticos con medicación oral. Estos resultados estaban en concordancia con estudios previos (Brenner et al., 2015; Savage et al., 1997). También se incluyeron en el estudio treinta y nueve voluntarios sanos sin enfermedad hepática. El criterio de exclusión para todos los grupos fue dolor crónico y/o agudo y cualquier signo de inflamación o lesión de la mano o pie dominante, que pudieran interferir con los resultados de la cuantificación sensorial. Todos los participantes fueron incluidos después de firmar un consentimiento informado por escrito. Los protocolos de estudio se ajustaron a las directrices éticas de la Declaración de Helsinki y fueron aprobados por los Comités de Ética e Investigación de ambos hospitales. Después de realizar las pruebas psicométricas, los pacientes fueron clasificados como: con o sin EHM (ver más abajo) y fueron remitidos a la Unidad de Neurofisiología para someterse a pruebas sensoriales, y electrofisiológicas. Las pruebas de biomecánica se realizaron en la Unidad de Evaluación de la Autonomía Personal, Dependencia y Trastorno Mental (TMAP) de la Universidad de Valencia. Estas pruebas se realizaron a la semana siguiente después del Psychometric

Hepatic Encephalopathy Score (PHES) y las otras pruebas psicométricas, con el fin de minimizar las posibles fluctuaciones cognitivas. La composición y características de los grupos se muestran en la Tabla 5.

1.2.1 Parámetros psicométricos

1.2.1.1 Psychometric Hepatic Encephalopathy Score (PHES)

El PHES es considerado como el "gold standard" es decir, la herramienta más eficaz para detectar EHM. Esta prueba consiste en una batería de pruebas psicométricas (Ferenci et al. 2002; Rudler et al., 2021; Weissenborn, 2015).

Se compone de cinco pruebas:

- Prueba de símbolos y dígitos (DST), evalúa la velocidad de procesamiento y la memoria de trabajo (Figura 6A).
- Prueba de conexión numérica-A (NCT-A), evalúa la atención y la velocidad de procesamiento (Figura 6B).
- Prueba de conexión numérica-B (NCT-B), también evalúa la atención y la velocidad de procesamiento (Figura 6C).
- Prueba de puntos en serie (SDT), evalúa la coordinación visomotora (Figura. 6D).
- La prueba de trazado de líneas (LTT), al igual que la SDT, examina la coordinación visomotora (Figura 6E).

La puntuación global del PHES se calculó y ajustó tanto por edad como por nivel de educación mediante tablas de normalidad españolas (http://www.redeh.org/TEST_phes.htm). Se consideró que los pacientes tenían EHM cuando la puntuación era igual o inferior a -4 puntos (Weissenborn, 2015).

1.2.1.2 Prueba STROOP

La prueba de Stroop se utilizó para evaluar la atención, la flexibilidad cognitiva y la resistencia a la interferencia (Stroop, 1935) (Figura 7). La prueba consta de tres partes. La primera es la tarea congruente, en la que se le presenta al sujeto una lista de nombres de colores, impresos en tinta negra. Entonces, el sujeto ha de leer tantas palabras como sea posible en 45 segundos. La segunda tarea, la tarea neutral, consiste en una lista de círculos impresos en diferentes colores y se le pide al participante que nombre tantos colores como sea posible en 45 segundos. La última tarea, llamada incongruente, consiste en una lista de nombres de colores (verde, azul, amarillo y rojo), impresos en una fuente de color diferente a la del nombre de color que se lee. El sujeto debe indicar el color de la fuente, no leer las palabras, tantas como sea posible en 45 segundos. En todas las tareas, cuando el sujeto llega al final de la lista antes de que se acabe el tiempo, vuelven a comenzar desde la parte superior de la lista. Se obtienen tres resultados directos (número máximo de ítems en cada parte), que fueron ajustados en relación con la edad y a partir de los cuales se calculó el parámetro de interferencia. Los valores obtenidos fueron interpolados a través de datos normativos de la población española (Golden, 2001)

1.2.1.3 Test d2

La prueba d2 evalúa la concentración, inhibición, atención selectiva y sostenida ya que se evalúa la capacidad de los sujetos para detectar estímulos relevantes e inhibir los irrelevantes (Figura 8). Los estímulos relevantes consisten en la letra "d" acompañada de dos pequeñas rayas (dos en la parte superior o inferior o una en cada parte). Los estímulos irrelevantes son el resto de ítems (todos las “p” independientemente de las rayas que tengan y las “d” con una, tres o cuatro rayas). El objetivo es marcar los ítems relevantes en una serie de 14 filas, empleando 20 segundos en cada fila. Se registran: las respuestas totales, el total de respuestas correctas, las omisiones, los errores, la efectividad total de la prueba y el índice de concentración e índice de variación. Todas las puntuaciones calculadas se transformaron a percentiles de acuerdo con las tablas de datos normativos (Brickenkamp, 2009; Giménez-Garzó et al., 2017).

1.2.1.4 Prueba oral de dígitos

El test oral lapso de dígitos evalúa la memoria inmediata y de trabajo (*Wechsler Adult Intelligence Scale*, WAIS) (Wechsler, 1955). Consta de dos partes: 'dígitos directos ' y 'dígitos inversos '. En los dígitos directos (Figura 9A), el sujeto debe repetir cada secuencia numérica, que va de tres a nueve elementos, en el mismo orden en que se le dijo (se permitieron dos ensayos para cada secuencia). La prueba continúa hasta que haya dos fallos consecutivos o cuando completa todas las secuencias. En los dígitos inversos (Figura 9B), las secuencias numéricas varían de dos a ocho elementos. Después de

escuchar la secuencia, el sujeto debe decir la secuencia en orden inverso. La prueba se continúa con los mismos criterios que los dígitos directos (Curran et. al, 2004).

1.2.1.5 Prueba oral de claves (SDMT oral)

La prueba oral de claves (SDMT, del inglés *Symbol Digit Modalities Test*) se utiliza para evaluar la atención y la velocidad de procesamiento (Ryan et al., 2020). Esta prueba consiste en una serie de nueve símbolos, en los que cada símbolo se empareja con un solo dígito, etiquetado del 1 al 9 (Smith, 1968). Los pacientes deben nombrar el dígito correcto asociado a cada símbolo con un tiempo de 90 segundos. Se registra el número de emparejamientos correctos y errores (Giménez-Garzó et al., 2017).

1.2.1.6 Test oral de letras y números

El test oral de letras y números de la batería WAIS (Egeland, 2015) mide la memoria de trabajo. Consiste en bloques de tres series que contienen letras y números mezclados (Figura 10). Después de escuchar una serie, el sujeto debe ordenar los elementos diciendo los números en orden ascendente y luego las letras ordenadas alfabéticamente. El número de elementos aumenta a medida que avanza la prueba. La prueba continúa hasta que el sujeto falla tres series. Se registran el total de respuestas correctas.

1.2.1.7 Pruebas coordinación motora

- Coordinación bimanual (Figura 11A): se le pide al sujeto que transfiriera todas las clavijas, de una parte del tablero a otra, utilizando ambas manos al mismo tiempo. Se registra el tiempo necesario para completar la prueba, la prueba se repitió dos veces en cada dirección. La prueba implica movimientos gruesos de las extremidades superiores y de destreza motora fina.
- Coordinación visomotora (Figura 11B): Consiste en una caja abierta, donde hay prismas metálicos en un lado y en el otro lado una placa metálica donde encajar los prismas, cada apertura tiene una orientación diferente. Usando la mano dominante, el sujeto debe colocar los prismas en orden, de izquierda a derecha, en las ranuras de la placa. La prueba se realiza dos veces y se registran el tiempo empleado (Felipo et al., 2012).

1.2.2 Parámetros biomecánicos

Se realizó una evaluación biomecánica del equilibrio, la marcha, la fuerza de la mano y la velocidad motora manual.

- Equilibrio: El equilibrio postural funcional fue evaluado mediante una plataforma de fuerza Dinascan/IBV y el sistema NedSVE®/IBV (vs. 5.1.0, 2013) (Instituto de Biomecánica de Valencia, Valencia, España). El sistema Ned/SVE evalúa el desplazamiento del centro de presión en tres ensayos de Romberg de 30 segundos bajo cuatro condiciones sensoriales: ojos abiertos o cerrados en una superficie firme, y ojos abiertos o cerrados en superficie inestable (espuma). Estudiamos los

resultados de la prueba de Romberg con la condición más inestable (ojos cerrados con los pies sobre un colchón de espuma): una puntuación del 100% reflejó la normalidad y otras puntuaciones reflejan discrepancias de los datos normativos emparejados por edad y altura proporcionados por el sistema (Urios et al., 2017).

- Evaluación de la marcha. La evaluación de la marcha se realizó con dos fotocélulas y dos plataformas de fuerza (Instituto Biomecánico Dinascan/IBV de Valencia, Valencia, España) y software NedAMH/IBV (versión 5.1.0, 2013, Instituto Biomecánico de Valencia, Valencia, España). Los participantes caminaron descalzos a lo largo de un corredor de 10 m de largo a una velocidad cómoda autoseleccionada. Las plataformas de fuerza se ubicaron en el centro del corredor para registrar el paso central y evitar la aceleración y desaceleración al inicio y al final del ciclo de marcha. El resultado registrado de la tarea de marcha fue la valoración global (%) y la velocidad de marcha (m/s).
- Fuerza de la mano: La fuerza de agarre es un marcador de sarcopenia, pérdida de masa y función del músculo esquelético relacionada con la edad. En este estudio se utilizó un dinamómetro electrónico isométrico (aplicación Ned Discapacidad/IBV con instrumento modulador de mano específico, Ned-VEP/IBV, Instituto Biomecánico Dinascan/IBV de Valencia, Valencia, España) para medir la fuerza tanto de la mano izquierda como de la derecha. El resultado de la contracción máxima se obtuvo a partir de la media de tres ensayos de fuerza. La fuerza de la mano se registró para tres posiciones funcionales durante 30 s de fuerza sostenida: (a) empuñadura, (b) pellizco lateral (pulgar y lado del dedo

índice), (c) pellizco de la punta (pulgar e índice). Se obtuvieron tres puntajes de fuerza máxima de ambas manos izquierda y derecha para cada posición funcional.

- Velocidad motora manual: medida por una tarea de velocidad de pulsación de botón. En la tarea de velocidad de pulsación de botón se utilizaron los dedos índices (derecho e izquierdo) y se midieron de forma independiente tres veces. La media de los tres ensayos se calculó para dar el resultado total, registrando así el resultado total de presiones por minuto para ambos dedos índices.

1.2.3 Parámetros bioquímicos

La extracción de sangre fue previa a las pruebas psicométricas y en el mismo día de la prueba PHES.

Los niveles de amonio se midieron inmediatamente tras la extracción de sangre y se analizó mediante el Ammonia Checker II® (Arkray Factory, Inc.), un dispositivo basado en la microdifusión, que cuantifica los niveles de amonio en sangre a partir de una muestra de 20 μL.

La interleucina 6 (IL-6) proinflamatoria se cuantificó mediante un kit ELISA (Thermo Scientific, USA) con un límite de detección de 1pg/mL.

1.2.4 Estudios neurofisiológicos

Todos los parámetros neurofisiológicos se estudiaron en el mismo día. Estos estudios permitieron la detección de la polineuropatía (PNP), su tipo y su patrón de

progresión. Estos datos permitieron estudiar la relación de la PNP con la enfermedad y el posible origen de los déficits sensoriales evaluados posteriormente. Cada laboratorio debe elaborar su propio protocolo independiente de evaluación neurofisiológica debido a las diferencias que existen entre los instrumentos y técnicas utilizados y las características individuales de la población de estudio.

1.2.4.1 Estudios neurofisiológicos de fibras de gran calibre: estudio de conducción nerviosa

El estudio de conducción nerviosa (ECN) evaluó la conducción nerviosa motora y sensitiva con Synergy, versión 22.0.0.144 y componentes: UltraPro S100 versión 1 y UltraProS100 DSP versión 591. El ECN motor se realizó en los siguientes nervios: cubital derecho, tibial derecho y peroneo derecho, y tibial izquierdo. El ECN sensitivo se realizó en los nervios peroneo (derecho e izquierdo), cubital y radial (izquierdo) y sural (derecho). Los parámetros medidos fueron: amplitud, latencia y velocidad de conducción. La latencia mide en milisegundos (ms) el tiempo de conducción nerviosa desde la aplicación del estímulo hasta el momento de la respuesta evocada. La amplitud es el valor medio, en milivoltios (mV), del pico negativo y el pico positivo de la respuesta evocada, evalúa el número de axones. Finalmente, la velocidad de conducción, expresada en m/s, se calculó midiendo la longitud entre dos puntos del mismo nervio y dividiéndola por el tiempo, que es la diferencia entre latencia proximal y latencia distal. Los datos obtenidos del ECN se utilizaron para detectar lesiones en las fibras nerviosas gruesas o de gran calibre, patología denominada como polineuropatía. El protocolo del ECN, utilizado para el diagnóstico de la polineuropatía, se basó en los descritos por Falck y Stålberg (Falck & Stålberg, 1997) y Preston y Shapiro (Preston & Shapiro, 2012)

1.2.4.2 Estudios neurofisiológicos de fibras de pequeño calibre: pruebas sensoriales cuantitativas (QST) y función autónoma

Prueba de cuantificación sensorial (QST)

La prueba de cuantificación sensorial (QST) se evaluó mediante el uso del CASE IV System WR Testworks (Figura 13A). Las modalidades de sensación medidas fueron; el Umbral de Detección Vibratoria (VDT) que evalúa fibras mielinizadas sensoriales de gran diámetro, A alfa; El umbral de detección de frío (CDT) permite medir fibras mielinizadas principalmente de diámetro pequeño, A delta; y el umbral de detección de dolor por calor (HPDT), que evalúa las fibras C no mielinizadas. El cálculo de estos umbrales sensoriales se realizó mediante la administración de una serie de estímulos vibratorios o térmicos no invasivos, correspondientes a un conjunto de 25 niveles estandarizados de estimulación vibratoria y térmica, de acuerdo con un algoritmo de "stepping" de un solo período de tiempo 4, 2, 1 (Dyck et. al, 1993). Este algoritmo determina cómo se presentan los estímulos. Durante una prueba dada, los estímulos posteriores dependen de la respuesta del paciente.

Estas pruebas tienen un total de 20 ensayos de estímulo; cada prueba corresponde a un período de tiempo. Durante este período de tiempo, el estímulo puede o no ser administrado (cinco períodos de estímulos nulos se colocan al azar para evitar resultados falsos). La luz verde señala el comienzo de un ensayo. Luego, se presenta un "1", que señala el período de tiempo de estímulo. El sujeto debe tratar de determinar si se administró o no un estímulo (vibratorio o térmico). Luego, el paciente responde presionando "sí" o "no" en el mando de respuesta (Figura 13E). En la prueba de dolor por

calor, el sujeto debe responder un número de 0 a 10, siendo 0 sin dolor y 10 siendo el dolor máximo posible.

Los estímulos VDT fueron administrados por el Estimulador de Vibración (Figura 13B) que consiste en un galvanómetro, establecido en 125 ciclos por segundo, variable entre 0 y 350 micrómetros.

Las pruebas CDT y HPDT se realizaron mediante un estimulador térmico (Figura 13C), que consiste en una placa de cerámica que produce una temperatura específica, que puede variar de 8.0 a 50.0 grados C, con una precisión de 1.25 a 0.25 grados C, en una superficie de 9.0 centímetros cuadrados (de acuerdo con los estándares del NIST, del inglés, National Institute of Standards and Technology). Antes de la prueba, el estimulador térmico se ajustó para que coincidiera con la temperatura basal de la piel del paciente. Para fines de normalización estadística, la temperatura basal fue de 30ºC para la prueba de frío y de 34 ºC para la prueba de calor-dolor. Para estímulos térmicos de alta magnitud, se agregó un tiempo de retención a la forma de onda, de modo que la temperatura absoluta se limita a 50ºC para calor y 8ºC para frío. Esta prolongación del tiempo del estímulo asegura la misma sensación fisiológica que una forma de onda piramidal más alta. Se realizaron un total de seis pruebas, 3 modalidades VDT, CDT y HPDT, primero en mano (Figura 13.2; 13.4) y luego en pie (Figura 13.1; 13.3). Los resultados fueron representados por los umbrales sensitivos de cada prueba individual en JND (del inglés, *Just Noticeable Difference*), correspondientes a la media del nivel de estímulos mínimos detectados por el sujeto durante la prueba. A partir de los datos recogidos del grupo control, se calculó un rango normal, que se utilizó para detectar pruebas fuera del rango normal para cada paciente individual, registrándose: el total de

pruebas en mano irregulares, total de pruebas en pie irregulares y el total de pruebas fuera del rango normal calculado. Finalmente, el tiempo total para completar cada prueba también se registró en segundos.

Función Autónoma

La respuesta simpática cutánea (RSC) explora el sistema nervioso autónomo por medio de cambios de potencial en la piel. Estos cambios en potencial se originan en las glándulas sudoríparas y la epidermis, en relación con la respuesta sudomotora y por lo tanto el sistema de termorregulación (Murota, 2016). Cuando el sistema nervioso autónomo se ve afectado por neuropatía de fibra fina (NFF), la latencia y la amplitud de RSC se alteran (Raasing et al., 2021). RSC, al igual que el estudio de conducción nerviosa, fue realizado por el Synergy UltraPro S100. Se colocó un electrodo de registro en la palma derecha y un electrodo de referencia en el dorso de la misma mano. Los electrodos utilizados eran comunes a la técnica EMG, con un filtro de bajas frecuencias de 0.5 Hz. El estímulo utilizado fue una pequeña estimulación eléctrica y los parámetros corresponden al ECN. La amplitud de la RSC se consideró anormal cuando era inferior a 1.1 mV. Este umbral se calculó a partir de la media de amplitud de RSC de los controles menos 2 desviaciones estándar.

Se evaluó la variabilidad de la frecuencia cardíaca (VFC) para detectar alteración en el sistema nervioso autónomo cardiovascular. Para ello se registró la frecuencia cardíaca en reposo y la variabilidad de la frecuencia cardíaca, que consistió en la variación del intervalo R-R, medido mediante electrocardiograma, en que se colocaron electrodos de registro en ambas muñecas encima de la arteria radial. La duración de los intervalos R-R

registrados reflejan la influencia de los sistemas nerviosos simpático y parasimpático en la modulación de la frecuencia cardíaca. Se realizaron diferentes maniobras de activación para la medición de VFC: la maniobra de Valsalva, hiperventilación y las pruebas ortostáticas (cambio postural desde decúbito supino a la sedestación) (Chémali & Chelimsky, 2014).

1.3 Análisis estadístico

Los valores se indican como la media ± el error estándar de media (SEM), a menos que se especifique lo contrario. Se utilizó la prueba de normalidad ómnibus de D'Agostino y Pearson para comprobar la normalidad de las variables. Las diferencias entre grupos se analizaron mediante ANOVA de una vía, seguido de la prueba de comparaciones múltiples post-hoc de Tukey. Para las variables no paramétricas, se realizó la prueba de Kruskal-Wallis, seguida de la prueba de comparaciones múltiples de Dunn. Los resultados fueron analizados mediante el programa GraphPad PRISM Versión 7. Las pruebas de coordinación bimanual y visomotora se analizaron mediante el análisis univariado de covarianza (ANCOVA) con la edad incluida como covariable, seguido de test Bonferroni para la comparación entre grupos. Los análisis de las tablas de contingencia se realizaron mediante la prueba exacta de Fisher. La capacidad predictiva de EHM para los parámetros de QST se calculó utilizando curvas ROC (*Receiver Operating Characteristic*). El análisis de correlación de Pearson y los análisis ROC se realizaron utilizando el programa SPSS, Versión 20 (SPSS Inc, Chicago, IL, USA) y los valores $p < 0.05$ se consideraron significativos.

1.4 Resultados

1.4.1 Clasificación de los pacientes mediante batería PHES

Los resultados de la puntuación PHES permitieron la clasificación de pacientes cirróticos en 38 pacientes sin EHM (NEHM) y 20 con EHM (Figura 14). La puntuación PHES para cada grupo se muestra en la Tabla 6. Los pacientes con EHM obtuvieron valores significativamente diferentes en comparación con controles ($p < 0.0001$) y pacientes sin EHM ($p < 0.0001$). No se encontraron diferencias entre los pacientes NEHM y controles sanos.

1.4.2 Pruebas neuropsicológicas

Se realizaron pruebas psicométricas adicionales para evaluar la atención selectiva y sostenida, la velocidad de procesamiento y la coordinación (Tabla 6). Las diferencias entre pacientes, con y sin EHM se encontraron en las pruebas de coordinación, prueba d2 excepto los valores de TA, prueba de Stroop y prueba de secuenciación de letras-números. En general, las pruebas neuropsicológicas se alteraron en ambos grupos de pacientes en comparación con los controles, con peores puntuaciones en pacientes con EHM que en pacientes NEHM.

1.4.3 Parámetros biomecánicos

Los resultados biomecánicos se representan en la Figura 15. Los resultados de equilibrio (Figura 15A) y la velocidad motora manual (Figura 15B) indicaron una diferencia significativa entre los pacientes con EHM y sin EHM ($p<0.05$). La velocidad de marcha era significativamente menor en los pacientes cirróticos con EHM que en los pacientes sin EHM ($p<0.01$). La valoración global de la marcha también fue significativamente menor en pacientes cirróticos con EHM que en los pacientes sin EHM ($p<0.05$) (Figura 15D). La fuerza de agarre no mostró diferencias significativas entre los pacientes (Figura 15E) ni la fuerza de los dedos, excepto para la pinza lateral izquierda, que sí mostró diferencias significativas entre NEHM y EHM (Figura 15 F).

1.4.4 Parámetros bioquímicos

Los niveles de amonio en sangre estaban aumentados en los dos grupos de pacientes con respecto al grupo control, no llegando a ser significativa la diferencia entre los pacientes sin y con EHM, aunque este grupo de pacientes presentaba una tendencia al aumento en los niveles de amonio con respecto a los pacientes NEHM ($p=0.067$) (Tabla 7; Figura 16).

Los niveles de IL-6 estaban aumentados significativamente en pacientes con EHM y eran significativamente más altos en comparación con los pacientes NEHM ($p<0.01$) (Tabla 7; Figura 16).

1.4.5 Estudio de conducción nerviosa

El estudio de conducción nerviosa (ECN) sensitiva y motora se muestra en la Figura 17. Los resultados del estudio de conducción nerviosa sensitiva muestran una amplitud reducida en pacientes cirróticos en todos los nervios evaluados (Figura 17A), mientras que la latencia aumentó en los nervios cubital y radial (Figura 17B). La velocidad de conducción de los nervios sensoriales se redujo en los nervios cubitales y radiales en los pacientes (Figura 17C). Se encontraron diferencias significativas entre los pacientes con y sin EHM (Figura 17B) en la latencia del nervio sural ($p<0.05$).

El ECN motor muestra una amplitud reducida en el nervio peroneo de los pacientes con EHM en comparación con los controles y el nervio tibial de los pacientes NEHM en comparación con los controles (Figura 17D). Los resultados indican un mayor retraso de latencia en ambos grupos de pacientes en los nervios cubital y peroneo (Figura 17E). La velocidad de conducción disminuyó en los grupos de pacientes en comparación con los controles (Figura 17F) y se encontraron diferencias significativas entre los pacientes con EHM y sin EHM en los nervios cubital y peroneo ($p<0.05$).

1.4.6 Estudio de fibras de pequeño calibre

El estudio de fibras de pequeño calibre se realizó mediante pruebas QST, RSC y VFC, los resultados se muestran en la Tabla 8.

Cuantificación Sensorial

Se encontraron umbrales sensoriales más altos en los dos grupos de pacientes en comparación con el grupo control para la prueba de QST en todas las modalidades, excepto para los pacientes NEHM en el umbral de dolor por calor 5.0 cuando se aplicó el estímulo en pie. Se encontraron diferencias significativas entre con y sin EHM en el umbral de detección de frío ($p = 0.04$), del dolor por calor 0.5 ($p = 0.01$) y el dolor por calor 5.0 ($p = 0.04$) en el pie (Tabla 8). La proporción de pacientes en los que más de la mitad de las pruebas estaban fuera del rango normal fue mayor ($p = 0.007$) en EHM (67%) que en pacientes NEHM (26%). Además, los pacientes tardaron más que los controles en terminar las pruebas en general. Los controles sanos terminaron cada prueba en alrededor de 120s, el grupo NEHM tardó un poco más, entre 125-180s y el grupo EHM más tiempo aún entre 130-220s. Los pacientes con EHM presentaban diferencias significativas con respecto a las NEHM en el tiempo de detección de vibración ($p = 0.04$), el tiempo de detección de enfriamiento ($p = 0.01$) y el tiempo de dolor por calor ($p = 0.01$), cuando el pie fue el sitio de prueba.

Función Autónoma

En cuanto a la función autónoma, hubo una disminución general en todos los parámetros de VFC (intervalo R-R), reduciéndose significativamente en los pacientes EHM en basal y siendo significativamente menor en prueba ortostática en pacientes EHM en comparación con sin EHM. La amplitud de la RSC se redujo significativamente en los

pacientes EHM en comparación con los controles ($p < 0.01$) y los pacientes NEHM ($p < 0.05$).

1.4.7 Estudio de conducción nerviosa de pacientes cirróticos con amplitud sural normal

Con el fin de detectar marcadores tempranos de EHM basados en parámetros neurofisiológicos, se seleccionó la amplitud del nervio sural como valor representativo del gran deterioro de la fibra sensitiva. Se eligió la amplitud del nervio sural ya que es el nervio más distal estudiado en miembros inferiores y uno de los primeros nervios afectados en las polineuropatías. Los valores de amplitud del nervio sural se consideraron normales o patológicos según los valores de referencia de la base de datos de nuestro laboratorio (corte: 15μV). La amplitud del nervio sural se vio afectada en el 42% de los pacientes NEHM y en el 40% de los pacientes EHM, lo que indica un deterioro de la fibra gruesa sensitiva

La Tabla 10 muestra los resultados de los estudios de conducción nerviosa en controles y pacientes con nervio sural normal. Los pacientes con EHM presentaron latencias más largas que los controles en los nervios cubital y radial. La latencia de la conducción nerviosa motora del cubital era mayor en los pacientes NEHM y EHM en comparación con los controles, mientras que las velocidades de conducción motora del cubital y peroneo estaban disminuidas en ambos grupos de pacientes en comparación con controles sanos, siendo significativamente menores en los pacientes EHM que pacientes NEHM en el cubital.

1.4.8 Resultados de QST de pacientes cirróticos con amplitud normal del nervio sural

La Tabla 11 resume el estudio de fibras de pequeño calibre en pacientes con amplitud sural normal. Los resultados en los test de detección de vibración y de frío mostraron niveles más altos de JND para los grupos EHM y NEHM en comparación con los controles, siendo aún más altos cuando el pie fue el lugar de prueba (Figura 18). Se encontraron diferencias significativas entre NEHM y EHM cuando el sitio de prueba fue en el pie en las modalidades de frío ($p=0.004$).

En cuanto a la detección de dolor por calor, los pacientes con EHM presentaban valores de JND significativamente mayores que los controles para la modalidad 0.5 de detección de dolor por calor, tanto en mano ($p<0.05$) como en pie ($p<0.01$), con una tendencia a ser significativamente mayor que en pacientes NEHM cuando el pie fue el sitio de prueba ($p=0.06$) (Tabla 11).

Se analizó la influencia de la etiología de cirrosis (alcohólica y de otro origen) en los resultados de QST en pacientes con amplitud normal del nervio sural (Tabla 12), así como la gravedad de la enfermedad hepática (Tabla 13) y la diabetes (Tabla 14). Se encontró que ninguno de estos factores influyó en los resultados de QST.

1.4.9 Función autónoma en pacientes cirróticos con amplitud normal del nervio sural

Los resultados de la RSC mostraron diferencias significativas entre los pacientes con y sin EHM en comparación con los controles tanto en latencia como en amplitud (Figura 17). Además, se encontraron diferencias entre pacientes, EHM y NEHM, en el valor de amplitud siendo significativamente menor para los pacientes con EHM ($p<0.03$) (Tabla 11).

La VFC también mostró diferencias entre los pacientes con y sin EHM en los resultados de las pruebas basales y ortostáticas (Figura 20). No se encontraron diferencias significativas entre los controles y los pacientes en ninguna de las pruebas de VFC, excepto en las basales, donde la variación del intervalo R-R de los pacientes con EHM fue significativamente menor.

Las pruebas de función autónoma se compararon entre los pacientes por etiología (Tabla 15), gravedad de la enfermedad hepática (Tabla 16) y presencia de diabetes (Tabla 17) no encontrándose diferencias significativas en ningún caso.

1.4.11 Correlación de los parámetros QST y función autónoma con las pruebas psicométricas y parámetros biomecánicos y bioquímicos

Se realizaron correlaciones entre los parámetros QST con las pruebas psicométricas (Tabla 18). El PHES, test estándar para la detección de EHM, correlacionó significativamente los umbrales de detección de frío en la mano y del dolor por calor 0.5

en el pie, así como con los tiempos de detección de frío en el pie y de la detección de vibración, tanto en la mano como en el pie. Estas correlaciones eran negativas, indicando que los pacientes con valores menores en la puntuación PHES presentaban mayores umbrales y tiempos de detección de los parámetros QST. La detección de frío en el pie, prueba de QST con más diferencias notables entre los grupos de pacientes, correlacionaba significativamente con la prueba Stroop, la Prueba d2, el test oral de claves y dígitos, y con los test de dígitos y de números y letras, pero no se correlacionó con el PHES o las pruebas de coordinación.

Se encontraron correlaciones significativas entre la amplitud de la respuesta simpática cutánea y el PHES ($r = 0.43$; $p = 0.02$), así como con las tareas congruente y neutra del Test Stroop ($r = 0.441$; $p = 0.027$ y $r = 0.51$; $p = 0.01$, respectivamente).

Los parámetros de QST también se correlacionaron con parámetros biomecánicos (Tabla 19). El tiempo de detección de vibración en la mano correlacionaba significativamente con la fuerza de la pinza lateral derecha e izquierda. El tiempo de HPDT para la mano correlacionaba significativamente con los resultados globales de la marcha, al igual que los umbrales sensitivos de VDT en la mano. Los resultados de CDT en el pie se correlacionaron con los resultados de agarre de fuerza de ambas manos y el umbral HPDT 5.0 en el pie correlacionaba con la fuerza de agarre en la mano derecha (Tabla 19).

Las correlaciones significativas de los parámetros QST y los niveles sanguíneos de IL-6 y amonio se muestran en la Tabla 20. Los niveles más altos de IL-6 se correlacionaron con umbrales de detección de enfriamiento más altos en el pie y en la

detección del dolor por calor 0.5 en la mano, como indican las correlaciones positivas significativas encontradas. También, los tiempos de detección más largos en las tres modalidades de QST se correlacionan con niveles más altos de IL-6 y amonio.

1.4.11 Capacidad predictiva de los test de cuantificación sensorial

Se realizó un análisis ROC para analizar la capacidad predictiva de los pacientes y los resultados mostraron que el umbral de detección de frío en el pie tiene una capacidad predictiva significativa para detectar EHM (Tabla 20; Figura 21), con la siguiente área bajo la curva ROC (AUC):

- Detección de frío en pie (JND): AUC: 0.759; 95% IC (0.565–0.952). La sensibilidad fue del 73% y la especificidad del 70%. Para un valor de corte de12.95 JND.

- Tiempo de detección de enfriamiento para pie (s): AUC: 0.838; IC del 95% (0.695–0.980). La sensibilidad fue del 75% y la especificidad del 68.4%. Para un valor de corte de 158.5 segundos.

1.5 Conclusiones

- Los pacientes con EHM muestran una disminución general de las capacidades cognitivas, motoras y sensoriales.
- El SNP se ve afectado de forma temprana en pacientes con EHM, una contribución novedosa, ya que la literatura previa determina que la alteración del SNP aparece en estadios tardíos de la encefalopatía hepática.
- La EHM en pacientes cirróticos está más asociada con alteraciones en la función de las fibras pequeñas (umbrales de dolor por enfriamiento y calor) que con la función de las fibras grandes (umbral de vibración).
- Las fibras finas del sistema nervioso autónomo y la sensibilidad al frío son las que se ven principalmente afectadas en EHM, de forma predominantemente distal y en extremidades inferiores, antes de que las fibras sensoriales gruesas se vean alteradas
- Hay una alteración temprana de la respuesta simpática cutánea en pacientes con EHM, con una menor prevalencia en pacientes sin EHM. Esto podría considerarse como un marcador temprano de fisiopatología que podría ser útil para la detección temprana de pacientes que son susceptibles de desarrollar EHM.
- Las alteraciones en sensibilidad térmica y mecánica se correlacionan con alteraciones en la atención, velocidad de procesamiento mental y memoria de trabajo en pacientes con cirrosis hepática.
- Las pruebas de cuantificación sensorial pueden ser útiles para detectar estos problemas desde el principio, así como la presencia de EHM, cuando aún se puede

intervenir para mejorar el pronóstico. Por tanto, esta detección temprana puede mejorar el resultado y la calidad de vida del paciente, permitiendo la implementación rápida de la intervención, lo que probablemente resultaría en una mejor respuesta a ésta debido a que la persona se encuentra en una etapa más temprana de la enfermedad.

- Las pruebas de cuantificación sensorial podrían ser una herramienta clínica práctica y funcional para utilizar como una prueba complementaria en la detección de EHM.

Index of abreviations

MHE Minimal Hepatic Encephalopathy

NAFLD Non-Alcoholic Fatty Liver Disease

HBV Hepatitis B virus

HCV Hepatitis C virus

HE Hepatic Encephalopathy

MELD Model for End Stage Liver Disease

LC Locus Coeruleus

DMN Default-Mode Network

TNFα Tumor Necrosis Factor Alpha

IL Interleukin

PNS Peripheral Nervous System

PNP Polyneuropathy

SFN Small Fibre Neuropathy

EMG Electromyography

PHES Psychometric Hepatic Encephalopathy Score

QST Quantitative Sensory Testing

NCS Nerve Conduction Study

VDT Vibratory Detection Threshold

CDT Cooling Detection Threshold

HPDT Heat Pain Detection Threshold

JND Just Noticeable Differences

SSR Sympathetic Skin Response

HRV Heart Rate Variability

ROC Receiver Operating Characteristic

II Abstract

II. Abstract

Cirrhotic patients may experience alterations in the peripheral nervous system and in somatosensory perception. Impairment of the somatosensory system could contribute to cognitive and motor alterations characteristic of minimal hepatic encephalopathy (MHE), which affects up to 40% of cirrhotic patients. We assessed the relationship between MHE and alterations in thermal, vibration, and/or Heat Pain sensitivity in 58 cirrhotic patients (38 without and 20 with MHE according to Psychometric Hepatic Encephalopathy Score) and 39 controls. All participants underwent attention and coordination tests, a nerve conduction study, autonomic function testing, and evaluation of sensory thresholds (vibration, cooling, and Heat Pain detection) by electromyography and quantitative sensory testing. The detection thresholds for cold and Heat Pain on the foot were higher in patients with MHE than those without MHE. This hyposensitivity correlated with attention deficits. Reaction times in the foot were longer in patients with MHE than without MHE. Patients with normal sural nerve amplitude showed altered thermal sensitivity and autonomic function, with stronger alterations in patients with MHE than in those without MHE. MHE patients show a general decrease in cognitive and sensory abilities. Small fibres of the autonomic nervous system and thermal sensitivity are altered early on in MHE, before large sensory fibres. Quantitative sensory testing could be used as a marker of MHE.

III Introduction

III. Introduction

3.1 Pathophysiology of hepatic encephalopathy

Chronic liver disease is a major cause of morbidity and mortality. Numerous conditions may lead to chronic liver disease, consequently leading to cirrhosis, and/or hepatocarcinoma, such as chronic hepatitis B virus (HBV), hepatitis C virus (HCV), non-alcoholic fatty liver disease (NAFLD), and alcohol-associated liver disease. Chronic hepatic damage from liver disease leads to fibrosis, a histological alteration of the liver in which perpetuation of normal wound-healing results in the accumulation of extracellular matrix proteins, forming a fibrous scar (Kisseleva & Brenner, 2021; Pinzani et al., 2011).

Cirrhosis is defined as an advanced stage of liver fibrosis that is accompanied by the distortion of hepatic vasculature (Schuppan & Afdhal, 2008). Up to 85% of cirrhotic patients may have minimal or covert hepatic encephalopathy (MHE), while 30-45% of patients with cirrhosis develop overt forms of hepatic encephalopathy (HE) (Ferenci et al., 2002). HE is a neuropsychiatric syndrome characterized by central nervous system dysfunction, due to alterations in the normal processes of the liver, such as filtration and hormone regulation. When these processes fail, toxins like ammonia, accumulate and filter into the circulatory system reaching the central nervous system. Development of HE is one of the complications that most impacts on the quality of life of patients and their caregivers (Bajaj et al., 2011; Montagnese & Bajaj, 2019).

Clinical features of HE can be classified into three different groups: the alteration of awareness level, neuropsychiatric symptoms, and neuromuscular signs (Cortés &

Córdoba, 2012). The pathogenesis of HE has not yet been fully elucidated. Although the ammonia poisoning theory has been core, the role of inflammatory mediators and other toxic substances have been receiving increasing attention (Aldridge et al., 2015). Currently, literature supports a synergic interaction between systemic inflammation and hyperammonaemia, in which both promote microglial activation, that lead to neuroinflammation and altered neurotransmission (Felipo et al., 2015).

3.1.1 The role of hyperammonemia in Hepatic Encephalopathy

Ammonia is a by-product of nitrogen metabolism. Under normal physiological conditions, ammonia derived from the gut is absorbed into the hepatic portal circulation and transported to the liver, where it is metabolized through the urea cycle. The liver is key in the homeostatic control of ammonia blood levels. When there is damage to the liver (acute or chronic) its ability to metabolize ammonia becomes impaired and can result in hyperammonaemia, an abnormally large amount of ammonia in blood. When the liver fails to metabolize ammonia, brain cells, such as astrocytes, and muscle cells, can act as an alternative metabolic pathway, metabolizing ammonia into glutamine through the glutamine synthetase enzyme (Aldridge et al., 2015; Rudler et al., 2021). This allostatic pathway carried out by astrocytes may protect the brain initially but eventually contributes to swelling and cytotoxic brain edema, leading to the development of cognitive impairment, which is potentially reversible with early on treatment (Cortés & Córdoba, 2012; Dhiman & Chawla, 2009).

Another contributing factor to astrocyte swelling is the presence of hyponatremia. Hyponatremia is present in 49,4% of cirrhotic patients (Angeli et al., 2006) and has been proposed to interact with hyperammonaemia, also promoting astrocyte dysfunction,

worsening prognosis considerably and accelerating progression of hepatic encephalopathy (Ginès & Guevara, 2008). Recent studies have demonstrated that even mild hyponatremia (sodium: 130-135 mEq/L) can lead to adaptative responses in the heart, bone, and brain systems but not without adverse effects. Hyponatremia can result in reversible gait abnormalities and subtle cognitive impairment, which have been found in seemingly asymptomatic chronic hyponatremia. It has also been associated with increased risk of falls (Portales-Castillo & Sterns, 2019). These clinical manifestations may also be found in cirrhotic patients in early stages of HE. Therefore, maintaining a sodium level greater than 135 mmol/L is essential, as it has also been associated with a lower response to therapeutic interventions such as lactulose treatment (Rudler et al., 2021).

Another theory proposes that there may be a vasogenic component to cerebral edema in acute liver failure. Although the exact mechanism of astrocyte swelling by ammonia has not yet been uncovered, the 'Trojan horse' hypothesis proposes that the excess of glutamine synthesized within astrocytes is transported into the mitochondria carrying ammonia with it, where it accumulates and can lead to oxidative stress and astrocyte swelling. Ammonia then interferes with the mitochondrial energy metabolism. Studies of in vitro and in vivo models of ammonia neurotoxicity have described an ATP depletion, in other words, cell exhaustion, related to the alteration of glutamine synthesis rather than the accumulation of glutamine (Aldridge et al., 2015).

3.1.2 The role of systemic inflammation in Hepatic Encephalopathy

Systemic inflammation can affect the function of somatic cells which further contributes to clinical manifestations present in cirrhotic patients. In cirrhosis, necrotic hepatic cells release DAMPs (damage associated molecular patterns), while the gut, which becomes more permeable (leaky gut), produces PAMPs (pathogen associated molecular patterns) and bacteria, stimulating the production of immune cells. Both patterns activate receptors such as toll-like receptors (TLR) and nod-like receptors (NLR). These innate immune sensors provide immediate responses against pathogenic invasion or tissue injury (Fukata et al., 2009), leading to the expression of immune cell surface molecules like adhesion molecules and cytokine receptors, promoting an increase of cytokines, chemokines, and growth factors, which recruit and activate additional inflammatory cells. In liver cirrhosis, pattern recognition receptor (PRR) dependent on immune cellular activation occurs locally in gut-associated lymphoid tissue (GALT), mesenteric lymph nodes (MLNs), and in peripheral blood. Immune cells already activated in GALT and MLN can reach circulation and systemically propagate the inflammatory response. As systemic inflammation increases, and cirrhosis becomes decompensated, overreactive immune responses become exhausted and eventually lead to immune deficiencies (Albillos et al., 2014). These changes in immunophenotype leading to MHE have been characterized (Figure 1), further pointing to peripheral inflammation as key in the progression of the disease and a possible cause of cerebral dysfunction (Mangas-Losada et al., 2017).

In summary, both ammonia and inflammation can lead to changes in the blood-brain barrier. Changes in blood-brain barrier can be disruptive (anatomical changes) or non-disruptive (functional changes). Whilst in HE the blood-brain barrier has been

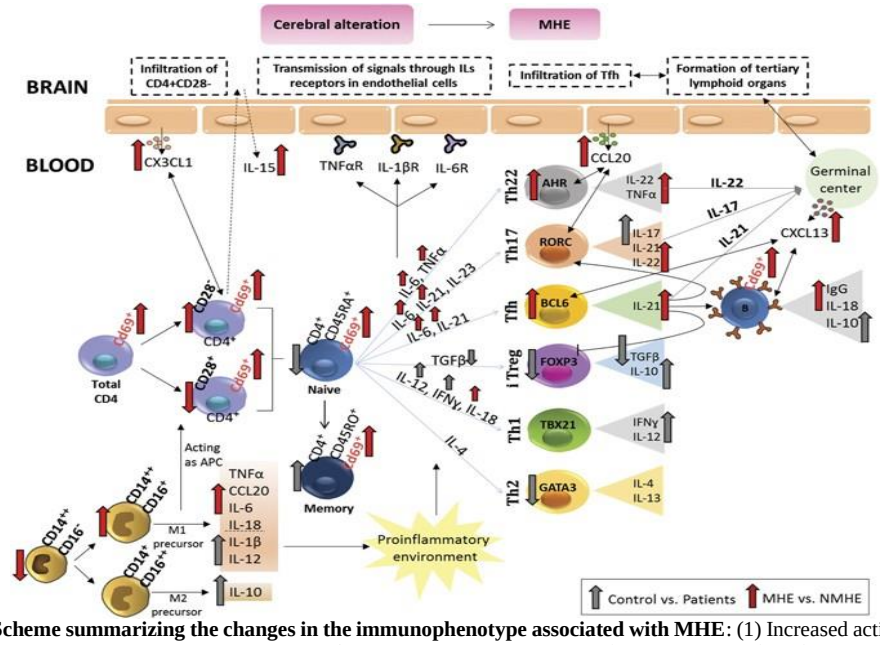

Figure 1. Scheme summarizing the changes in the immunophenotype associated with MHE: (1) Increased activation of subtypes of CD4+ T-lymphocytes, by the over expression of CD69. (2) Increased amount of CD4+CD28− T lymphocytes, are associated with higher levels of CX3CL1 (fractalkine) and of IL-15. (3) Increase in differentiation of CD4+ T lymphocytes to Th follicular and Th22. (4) Increased activation of B lymphocytes and serum IgG. These four main alterations may contribute separately or jointly to alter cerebral function and to the appearance of the neurological alterations associated to MHE in cirrhotic patients. Some possible mechanisms by which changes in peripheral inflammation in patients with MHE may contribute to the appearance of the neurological alterations are: (a) infiltration into the brain of CD4+CD28− T lymphocytes, leading to neuroinflammation and neurological impairment; (b) activation by peripheral interleukins (TNFα, IL-1b, IL-6) of their receptors in endothelial cells, triggering the release of inflammatory factors into the brain, neuroinflammation and neurological alterations; (c) infiltration of Tfh cells and formation of tertiary lymphoid organs with B lymphocytes germinal centres, leading to neurological alterations. From: Mangas-Losada et al., 2017.

shown not to alter anatomically, functional changes do occur, leading to a wide range of clinical manifestations (Wright et al., 2007).

3.2 Hepatic encephalopathy diagnosis and clinical manifestations

The scale most often used for grading the extent of HE is the West Haven criteria, which distinguishes between four grades of clinically overt HE. Currently, experts suggest differentiating between covert HE (MHE plus grade I HE according to West Haven criteria) and overt HE (grades II–IV) (Vilstrup et al., 2014). Table 1 indicates the different stages of HE, classified by the West Haven criteria, with the corresponding clinical manifestations of cirrhotic patients in each stage.

Table 1. West Haven Criteria for the classification of different stages of HE.

	Stage of HE	*Clinical manifestations*
Covert Hepatic Encephalopathy (CHE)	Minimal	Subtle motor and cognitive deficits related to attention, speed, and executive functions without clinical evidence of mental change.
	I	Trivial lack of awareness Euphoria or anxiety Shortened attention span Impairment of addition or subtraction Altered sleep rhythm
Overt Hepatic Encephalopathy (OHE)	II	Lethargy or apathy Disorientation for time Obvious personality change Inappropriate behaviour Dyspraxia Asterixis
	III	Somnolence to semi-stupor Responsive to stimuli Confused Gross disorientation Bizarre behaviour
	IV	Coma

The severity of liver damage is assessed by the Child-Pugh score (Table 2) and the MELD (Model for End Stage Liver Disease) score, which are both based on clinical parameters. The Child-Pugh score classifies patients into three categories; A: good hepatic function; B: moderately impaired hepatic function; and C: advanced hepatic dysfunction.

Table 2. Child-Pugh Classification [a]

	1 point	2 points	3 points
Bilirubin (mg/dl)	< 2	2-3	>3
Albumin (g/dl)	> 3.5	2.8-3.5	< 2.8
PT[b] prolongation (s)	1-3	4-6	>6
Ascites	None	Mild	Refractory
Encephalopathy	None	Mild (grades 1-2)	Severe (grades 3-4)

[a] Child classes: A, 5-6 points; B 7-9 points; C, 10-15 points. [b] PT, prothrombin time

Frequently, more than one scale is used as each have their own benefits and limitations. For example, the MELD score is more accurate than the Child-Pugh when predicting short-term outcome after transjugular intrahepatic portosystemic shunt (TIPS) but is less accurate for long-term predictions (Botta et al., 2003; Salerno et al., 2002).

The MELD score is calculated from blood creatinine, bilirubin and INR levels, with the following formula: MELD Score = 9.6 Ln(Creatinine)+3.8 Ln(Br) + 11.2 Ln(INR) + 6.4. In 2006, this formula was modified to include blood sodium levels, as it was demonstrated to be an important criteria in the prediction of mortality (Biggins et al., 2006), further supporting the idea of hyponatremia interacting with hyperammonaemia. The formula was defined as the MELD Na Score = MELD - Na-(0.025*MELD*(140-Na)) +140. It is important to clarify that the maximum value for creatinine is 4 and if the patient has undergone dialysis twice in the week before examination, the maximum value must be used.

3.3 The challenges of grading the extent of Hepatic Encephalopathy

Currently, there is a large group, 30%-50% of cirrhotic patients, that present what is defined as minimal hepatic encephalopathy (MHE). MHE patients do not show clinically overt signs of HE but they do show mild motor and cognitive impairments. These impairments are not detected through traditional clinical methods but are capable of impairing health related quality of life and the participation in daily life activities such as driving and working (Dhiman et al., 2010; Felipo et al., 2013; Rudler et al., 2021). MHE is currently detected by the Psychometric Hepatic Encephalopathy Score (PHES), a battery of psychometric tests. Although these psychometric tests are a first-line tool for

diagnosing MHE, recent studies indicate there is still a percentage of patients who cannot benefit from early detection through this method (Giménez-Garzó et al., 2017). MHE patients also have a higher risk of traffic, home, and work accidents. They are more prone to suffer falls, fractures, and hospitalizations, which pose a high economic burden on health systems (Hirode et al., 2019). Therefore, MHE has an important impact on patient's daily life, making daily activities become more difficult and worsening patients' level of autonomy, independence, and quality of life (Stinton & Jayakumar, 2013).

3.4 The somatosensory system

The somatosensory system is a component of the nervous system that detects and allows perception of pain, temperature, head and body position, movement, and touch (Jacobs, 2011). The skin plays a core role in this system, being as it were, a complex organ, with the ability to maintain internal homeostasis by way of multidirectional communications between the endocrine, immune, and central nervous systems, by means of neuropeptides, cytokines, hormones, and other effector molecules (Brazzini et al., 2003). It is the first line of defence, as well as the most fundamental way in which we interact with the exterior world. Yet, there is still no standard practice for checking its correct functioning, but vision and hearing examinations are common in daily clinical practice.

There are already many studies that argue and support that evaluating function of the different senses can indicate changes in the central nervous system. For example; the loss of smell, anosmia, as a predictor of neurodegenerative diseases (Albers et al., 2006);

or the function of the visual system, which is intimately related to attention and aids in the diagnosis of neurological damage (Das et al., 2007).

The function of the somatosensory system is of interest in MHE for various reasons. Due to the implication of this particular system in awareness, attention and motor response (Picard & Friston, 2014), it is clearly related to all clinical symptoms that can be observed in HE. Also, reports suggest a reduced variability in brain responses across individuals in sensory tasks compared to cognitive tasks, making it a possible sensitive marker for cognitive decline (Stephen et al., 2010). Diseases that may also present mild cognitive impairment such as, diabetes and Parkinson disease, have been demonstrated to have sensory deficits (Nolano et al., 2008). Additionally, Brenner demonstrated through neurophysiological methods that cirrhotic patients with advanced HE, show impairments of both central and peripheral parts of the somatosensory system (Brenner et al., 2015).

3.4.1 Level of awareness, Attention, and Sensory Processing

Somatosensory processing is performed by a complex interaction between different parietal cortical regions and the thalamus, in intimate relation to other sensory systems. Mountcastle (2005) subdivides somatosensory function into large- and small-fibered. The large-fibered somatic afferent system includes the discriminative-sensory systems which involve the dorsal and dorsolateral columns and the trigeminal leminiscal system that manage mechanoreceptive aspects of sensation. The small-fibred system involves affective-vegetative systems, which relay all somatosensory information from the spinal columns and the spinal trigeminothalamic tract, and converges with the nuclei of discriminative-sensory systems, also having individual channels to frontal, lateral and

limbic cortical circuits to deal with emotion and effect reactions (ten Donkelaar et al., 2020).

These affective-vegetative systems include interoceptive information which direct efferent brain-to-body control of physiology to maintain health and integrate sensory, emotional, cognitive, and motivational representations (Bonaz et al., 2021). This information is key in homeostasis, regulation of level of awareness, attention and adequately adapting responses to the demands of the environment.

The ability to regulate sensory signal processing to attend to behavioural demands is intimately related to maintaining adequate indices of arousal and awareness, in which the locus coeruleus - norepinephrine (LC-NE) system is key. LC neurons are especially vulnerable to toxins and infection because of its high exposure to blood circulation compared to other parts of the brain. In addition, it is close to the fourth ventricle and therefore may also be exposed to toxins in cerebrospinal fluid. Lower LC neural density has been associated with cognitive decline and Alzheimer's disease, where tau pathology precursor emerges in the LC by early adulthood in most people (Mather & Harley, 2016). In MHE, focal cortical damage has been found parallel to cognitive impairment in brain areas such as, the superior temporal lobe and precuneus (Montoliu et al., 2012).

The superior temporal lobe is related to auditory processing, social cognition and analysing information about location of visual stimuli (Waxman, 2013). The precuneus, is involved in a variety of functions including recollection and memory, integration of information relating to perception of the environment, cue reactivity, mental imagery strategies, episodic memory retrieval, and affective responses to pain (Borsook et al., 2015). A study by Utevsky et al. (2014), indicates that the precuneus also plays a core

role in the default-mode network (DMN), a network of interacting brain regions that activates when a person is not focused on the outside world (Utevsky et al., 2014).

The LC-NE system is involved in the modulation of sleep-wake cycles, facilitation of attention, responses to stress, initiation memory formation, and retrieval. It modulates blood flow, metabolism, and the distribution of oxygen and glucose throughout the brain. Therefore, this system has also been studied as a potential neural modulator of the DMN. Various studies show that mind wandering results in loss of sensitivity to sensory stimuli (Mittner et al., 2016). This loss of sensitivity has an inverted-U relationship (Figure 2) in which changes in neural responsiveness correspond to tonic and phasic modes of LC discharge in a spectrum of waking behavioural states (Waterhouse & Navarra, 2019).

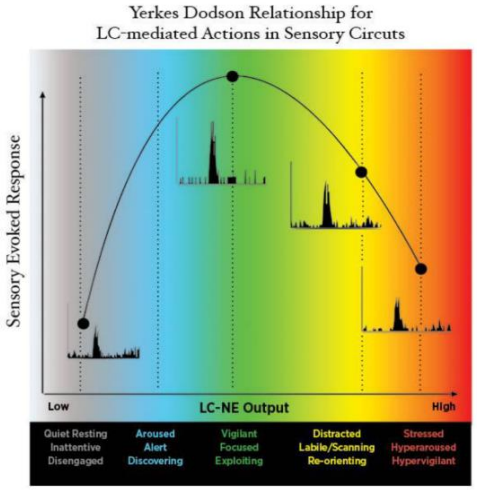

Figure 2. Yerkes Dodson relationship for LC-mediated actions in sensory circuits. Each waking state corresponds to a LC-NE output which varies from low when arousal is low to high when arousal is high. When a stimulus is presented, the sensory evoked response is perceived on the basis of this LC-NE output in an inverted U like manner. The sensory evoked response is low when alertness is low parallel to LC-NE output which is low, when alertness and LC-NE output increase the sensory evoked response is much larger, when alertness is above a functional level and the LC-NE output is high the sensory evoked response decreases. Image from: Waterhouse et al., 2019.

Recent neuroimaging studies have also increased our knowledge on brain-gut interaction. Altered gastrointestinal function and altered brain activity can be found, especially in association with emotional response (Kano et al., 2018). Gastrointestinal stimulation activates regions associated with sensor processing (Figure 3) including the

salience network (SN) which is involved in detecting, filtering, and determining the importance of external and interoceptive stimuli, and moderates physiological arousal via its association with the amygdala (Menon & Uddin, 2010). In MHE patients it has been shown that there is a reduction in resting-state functional connectivity in the DMN and reduced grey matter in the right frontal lobe, right insula, and right cerebellum. These alterations correlated with the scores of different cognitive tests (García-García et al., 2017).

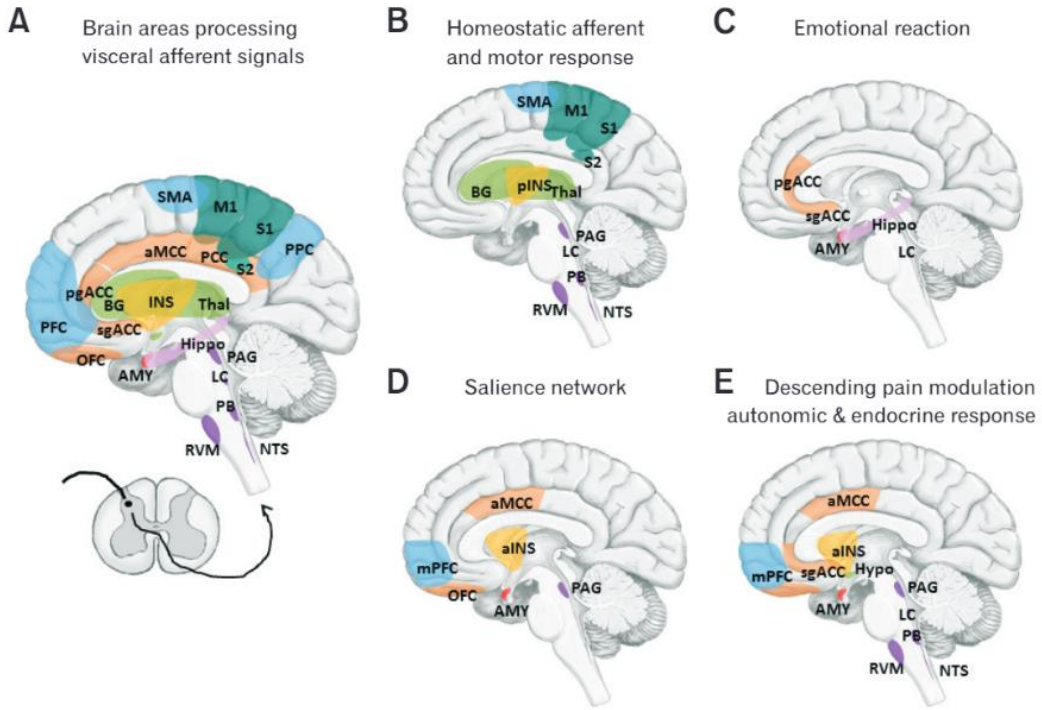

Figure 3. Brain areas processing visceral afferent signals and the areas altered in functional gastrointestinal disorders.(A) Afferent visceral signals convey from the gut projecting the nucleus of the solitary tract (NTS) and the lamina I of the dorsal horn and are integrated in the parabrachial nucleus (PB) in the brainstem and routed to the thalamus (Thal), where 2 parallel streams of information reach the insula (INS) and the anterior cingulate cortices (ACC). (B) Homeostatic afferent: the brain areas related to sensory processing of homeostatic condition of the gut, brainstem sensory nuclei (NST and PB), Thal, posterior insula (pINS), and somatosensory cortex (S1 and S2). The basal ganglia (BG), supplementary motor cortex (SMA), primary motor cortex (M1), brainstem nucleus (periaqueductal gray [PAG], locus coeruleus [LC], and rostroventral medulla [RVM]) are associated with the preparation of the reaction and motor response to afferent signals. (C) Areas association with emotional arousal reactions: LC, amygdala (AMY), subgenual and pregenual ACC (sgACC and pgACC), medial prefrontal cortex (mPFC), and hippocampus (Hippo). (D) Salience network: network is engaged in response to salient stimuli but no limited to pain. Core region of salience network is the anterior insula (aINS) and anterior midcingulate cortex (aMCC). The midcingulate cortex, part of the dorsal ACC, is a multifunction region involved in the executive control of attention. The aINS is essential for the conscious experience (bodily) feelings. (E) Descending pain modulation system: endogenous descending pain modulation structures include PAG and the RVM, which receives direct nociceptive information from the PB and the spinoreticular pathway and top-down modulation from the prefrontal executive control areas (mPFC and dorsolateral PFC), aMCC and emotional arousal areas (aINS, sgACC and AMY) and modulates the sensitivity of spinal dorsal horn neurons. These areas are highly connected with autonomic and endocrine response structures (hypothalamus [Hypo] and brainstem nucleus including NTS). Image from: Kano et al., 2018.

In summary, the necessary level of awareness and attention, in other words up-down signalling, is essential for the modulation and integration of sensory input (down-up signalling), just as much as down-up signalling is necessary for regulating homeostatic processes (Gomez-Ramirez et al., 2016), which in effect regulates level of awareness. Therefore, awareness, attention and sensory processing are intimately related as a result, when there is an alteration in one of these components it may alter the function of the others.

3.4.2 Thermoregulation, Sensory processing, and the Immune System

Thermoregulation is essential to survival and plays a fundamental part in keeping homeostasis. It is intrinsically related with the somatosensory system even before birth. Having common receptors and effectors which play a major part in functionally responding to environmental demands and internal homeostasis.

In humans, body temperature is typically high when compared to other animals and is relatively independent of environmental conditions, metabolism is fast, and the main source of thermal energy is produced by internal body processes where liver metabolism and brown adipose tissue play a crucial part in thermogenesis (Thorne et al., 2020). When defining temperature, it is important to distinguish between core temperature which corresponds to the thoracic, abdominal viscera and the brain, and shell temperature which surrounds the core. Core temperature in humans is generally very stable and can expand and contract through redistribution of blood. Expansion of heat takes place when blood from the abdominal viscera is redistributed to the skin. Many thermoregulation mechanisms have been studied in detail in rat, such as hypothermia, as

a cause of dementia, but little is known about these mechanisms in humans. Shell temperature, on the other hand, can vary widely. The outside layer of the shell is the skin, which is rich in thermoreceptors and common for the perception of heat, cold and pain. Therefore, the skin plays a crucial part in thermoregulation and somatosensory systems. Different skin types, glabrous and non-glabrous, have different roles. Glabrous skin, like the human palm, is densely vascularised and has two opposite thermoeffector responses: 1. Rapid heat dumping "the radiator effect" which occurs when cutaneous vasodilatation increases and is directed to anastomoses. 2. Abrupt interruption of heat loss as anastomoses shut in vasoconstriction. Therefore, glabrous skin primarily reflects vasomotor tone (Romanovsky, 2018).

Thermoregulation has long been known to be associated to the immune system, where both fever and hypothermic responses are considered to be evolved strategies that optimize defences in disease, even though they are energetically costly (Childs, 2018). Fever, for example, leads to muscle atrophy mechanisms, a process which is set on by many factors such as the nature of the primary disease, the levels of proinflammatory cytokines such as TNFα, IL- 1β and IL-6, anorexic response, hormones, metabolism and pathogen factors (Garami et al., 2018; Schieber & Ayres, 2016). A similar mechanism has been associated with cirrhotic patients who can develop sarcopenia, a severe muscle depletion (Ponziani et al., 2021).

Furthermore, it has been found that skin temperature variability is an independent predictor of survival in cirrhotic patients (Bottaro et al., 2020). In addition, these patients have been shown to exhibit higher distal temperature values and blunted rhythmicity compared to healthy controls, which in turn correlate with sleep-wake disturbances

(Garrido et al., 2017). Therefore, thermoregulation seems to play an important role in the pathophysiology of cirrhosis. Likewise, deficiencies in thermoregulation in cirrhotic patients seem to be intimately related to circadian rhythm misalignment also present in these patients. This circadian rhythm alteration can cause a variety of sleep disorders even in the absence of neurocognitive deficits associated with hepatic encephalopathy (HE) (Plotogea et al., 2021). Moreover, impaired thermoregulation with high distal skin temperature may be related with portal hypertension and hyperdynamic circulation (Bolognesi et al., 2014).

The central mechanism of thermoperception remains elusive, especially when referring to cooling sensation. It has been shown that cutaneous thermosensory activity relates to a somatotopic map in the dorsal posterior insular cortex (Craig, 2018), which originates in lamina I of the superficial dorsal horn and ascends by way of the lateral spinothalamic tract and to a distinct region in posterolateral thalamus. The insular cortex is the primary cortical substrate for interoceptive representation that drives the central autonomic network and has been linked to thermosensation. central autonomic network orchestrates autonomic, endocrine, motor and behavioural responses such as stress and immune reactivity in order to adapt to uncertain external environments (Bonaz et al., 2021). A study by García-García et al. (2017), found atrophy in the grey matter of the insula of MHE patients which correlated with cognitive alterations. These grey matter abnormalities in the insula were found to have predictive capacity for the detection of MHE and correlated with cognitive alterations, increases in serum IL-6 and MELD score.

3.5 The peripheral nervous system

The peripheral nervous system (PNS) is a vast network by which the central nervous system communicates with all other organ systems in the body. The PNS carries sensory information regarding internal and external environmental conditions and motor signals to control the activities of cells, visceral organs, and musculoskeletal system. It is divided into the somatic PNS and autonomic PNS.

The somatic PNS arises in the dorsal root ganglia which are located along the vertebral column and harbour sensory neurons with large nuclei and prominent nucleoli.

The ganglia of the autonomic PNS are widely dispersed throughout the body. The autonomic PNS sympathetic ganglia are typically positioned at a distance from innervated organs and the autonomic PNS parasympathetic ganglia are located within the walls of innervated organs.

3.5.1 Cutaneous sensory system, fibres and pathways

The cutaneous sensory receptors reside in the structures of the dermis and transduce stimuli into neural impulses. These can be classified into mechanoreceptors, thermoreceptors, and nociceptors. Evolution has promoted specialization of the different senses and therefore also the physiology of the skin, but some senses remain unmodified due to its protective function. Heat detection and pain are an example of this. The sensory receptors (Table 3) that mediate the perception of temperature and pain are the simplest, the “free” nerve endings (Dellon, 2016). Heat-pain is transmitted in two phases, first by small diameter myelinated A delta fibres and unmyelinated C fibres (Figure 4). As A delta fibres are quicker than unmyelinated C fibres, pain is phasic in nature, even though the

stimulus is only applied once. Cooling perception is mainly relayed by small diameter myelinated fibres (A delta). Finally, vibration travels through large diameter sensory myelinated fibres (A alpha). All this data is conducted through afferent fibres of pseudounipolar neurons of the spinal ganglion, reaching the posterior spinal medulla, where crossing may take place directly (protopathic sensibility: crude tactile sensations of temperature, pressure, pain) or further on, at the brain stem (epicritic sensibility: discriminative sensations of fine touch and temperature). Lastly, the afferents end in a contralateral manner to the location of the received stimulus, in the corresponding somatotopic region of the cortex.

Table 3. Classification of sensory receptors

Sensory Receptor	General response	Nerve ending type	Stimuli
Mechanoreceptors	Physical changes including touch, pressure, vibration and stretch	Pacinian corpuscles	Vibration
		Meissner corpuscles	Pressure
		Merkel complexes	Structure and texture
		Ruffini corpuscles	Stretch
		C-fibre LTM (low threshold mechanoreceptor)	Pleasant, light tactile sensations
		Hair follicle related mechanoreceptor	Light touch
	Proprioceptors	Muscle spindles	Muscle contraction / relaxation
		Golgi tendon	Muscle strain
Thermoreceptors	Temperature changes	Ruffini corpuscles	Heat
		Krause corpuscle	Cold
Nociceptors	Pain related to temperature, pressure, and chemicals.	Free nerve endings	Noxious heat Noxious cold Noxious pressure

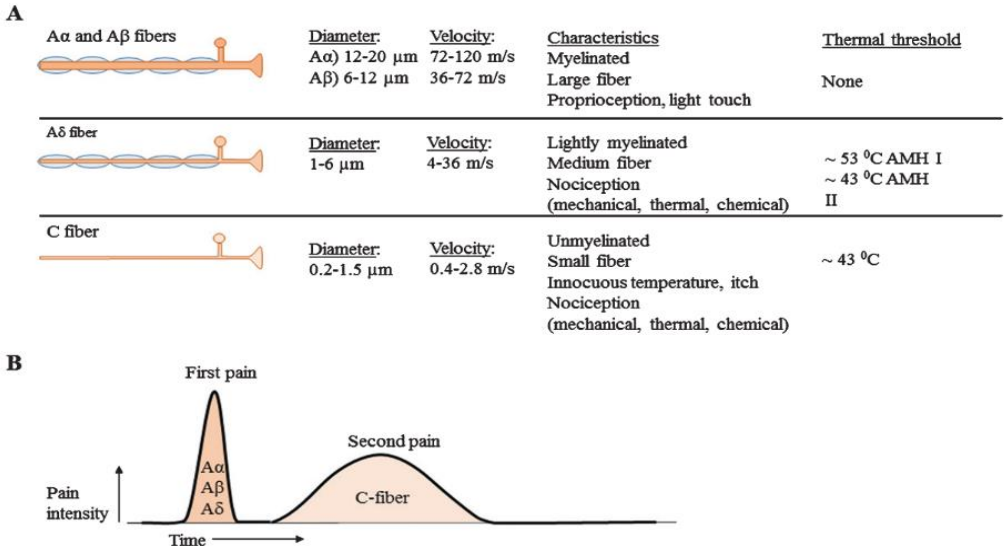

Figure 4. Small nerve fibres classification. (A) and small nerve fibres response to pain (B) Figure from: Raasing et al., 2021.

3.5.2 Neuropathies

Neuropathy is defined as the damage to the nerves that connect the spinal cord to muscles, skin, blood vessels and organs (Dellon, 2016). Neuropathies can be classified in many ways but in clinical settings when the origin is not genetic or specific, such is the case of Charcot-Marie-Tooth disease, Guillain-Barré syndrome or carpal tunnel syndrome, they can be generically classified into polyneuropathy (PNP), affecting two or more nerves and mononeuropathy, affecting only one nerve. Depending on the diameter of nerves affected, neuropathies may be classified into large fibre neuropathy and small fibre neuropathy (SFN). Several nerve conduction studies have shown that up to 70% of cirrhotic patients could be affected by polyneuropathy (Chaudhry et al., 1999; Hoitsma et al., 2004).

3.5.2.1 Polyneuropathies

Polyneuropathy is a peripheral neuropathy characterized by symmetrical sensory symptoms, such as numbness, paresthesia and pain, and muscle weakness (Hanewinckel et al., 2016). It is the most common disorder of the peripheral nervous system (Sommer et al., 2018) being a symptom or complication of a wide range of diseases, such as diabetes and end-stage renal failure, especially when treatment is started too late. PNPs are mainly diagnosed by review of patient history and neurophysiology testing such as neurography and electromyography (EMG), as well as evoked potentials (Said, 2013). PNP in some cases may involve in small fibre damage.

3.5.2.2 Large fibre neuropathy

Large myelinated fibres permit coordination of motor orders and sensory feedback from muscle proprioceptors and joint position sensors, this enables rapid and precise muscle action which allow for the accomplishment motor goals (Chew et al., 2020). This is why large fibre neuropathy manifests with the loss of joint position and vibration sense and sensory ataxia. Therefore, large myelinated fibres carry sensory and motor information that both must be evaluated. Electromyography and nerve conduction studies are well established neurophysiologic techniques that are used to assess the integrity of larger myelinated sensory and motor fibres (Hovaguimian & Gibbons, 2012).

3.5.2.3 Small fibre neuropathy

Small fibre neuropathy is the disorder of the small myelinated Aδ-fibres and unmyelinated C-fibres. SFN may affect small sensory fibres, autonomic fibres or both, producing changes in sensory and autonomic function. Small somatic nerve fibres transmit information about temperature, pain and itch, whilst small autonomic nerve fibres are responsible for thermoregulatory, sudomotor, cardiovascular, gastrointestinal, urogenital and other autonomic functions (Raasing et al., 2021). Sudomotor fibres innervating sweat glands are cholinergic, postganglionic, sympathetic, and unmyelinated. They travel with other peripheral nerves. Their preganglionic fibres originate in the spinal cord intermediolateral cell column, exit in the white rami, and synapse in the paravertebral sympathetic ganglia (Lacomis, 2002). The clinical presentation of SFN is diverse and no single clinical pattern fits all manifestations. The most common presentations of SFN are length-dependent polyneuropathy and non-length-dependent ganglionopathy (Figure 5).

Patients with length-dependent SFN (Figure 5A) can present neuropathic pain in the feet, frequently burning pain. However, the condition can also be pain free, with reduced or no pain or temperature sensation. Alterations follow a distal-to-proximal pattern beginning in the extremities (toes and feet), reaching the ankles and even the knees. Once symptoms are at the knee, the upper limbs can become involved and, consistent with the length-dependent topography, the fingertips are the first involved. This pattern is typically found in diabetic patients and other metabolic syndromes.

Non-length-dependent SFN (Figure 5B) is characterised by signs or symptoms caused by the functional impairment of individual or multiple nerve fibres. This pattern

has been described in paraneoplastic, immune-mediated, and idiopathic cases (Terkelsen et al., 2017).

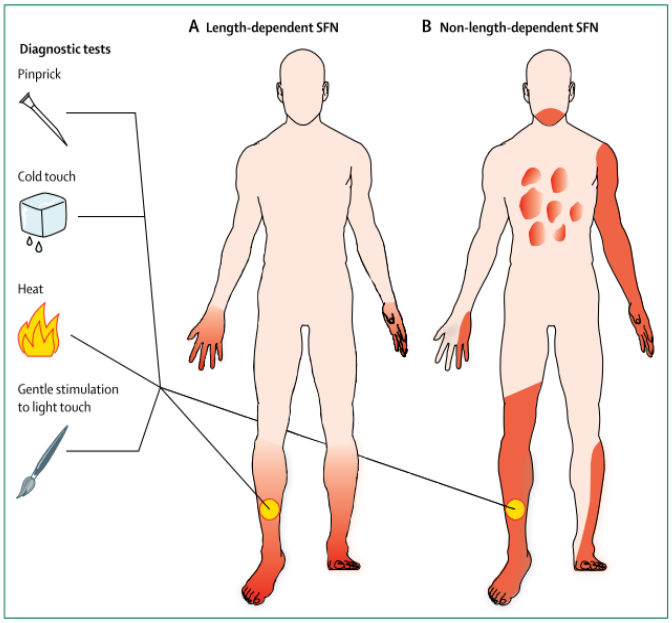

Figure 5. Clinical presentations in small fibre neuropathy (SFN). (A) Length-dependent SFN where pain, sensory loss or hypersensitivity may manifest in a characteristic stocking-glove distribution, with intact deep tendon reflexes and preserved proprioception and sensation to vibration. (B) Non-length-dependent neuropathy may manifest reduced or increased small fibre function corresponding to a single or multiple nerves. Figure from: (Terkelsen et al., 2017)

Inflammatory and autoreactive conditions are increasingly linked to SFN, unsurprising as small nerve fibres are implicated in immunity from the moment of gestation, when their cell bodies leave the protection of the central nervous system to become sentinels and first responders. This sensory ganglion develops fenestrated capillaries and terminal distal C-fibres that nearly contact the exterior world, inserting itself into the superficial epidermis and mucosa. In addition, small fibres have receptors for the release of immune or inflammatory signals in the most distal parts of the body. They also contact mast cells and microvessels. Neurogenic inflammation mediated by C-fibres can sometimes be visible as flushing and swelling in focal or generalized small-

fibre neuropathy (Figure 2A and C). Evidence suggests that SFN can be associated with dysimmunity and can improve with immunomodulatory therapies (Oaklander & Nolano, 2019). Diagnosing SFN can be challenging and quantitative sensory testing, quantitative sudomotor function and skin biopsy are techniques that can be used, but physical examination findings are still considered the gold standard against which all tests are compared when making a diagnosis of SFN (Hovaguimian & Gibbons, 2012).

3.6 Evaluating function of cutaneous sensation

Evaluating sensation is a fundamental part of a standard neurological exam although seemingly simple in technique it can be subjective, therefore must be performed carefully and with full patient cooperation (Bigley, 1990). Sensory alterations impact the way we relate to the world. Consequently, they can lead to deficits in motor function and altered pain perception, both fundamental to carrying out daily life activities and enabling individual's autonomy.

3.6.1 Traditional methods

Quantification of sensation started being applied in the 60's, primarily through the efforts of Moberg in hand surgery (E Moberg, 1958, 1990; Erik Moberg, 1964), and later on by Buford (W L Jr Buford, 1995; W L Jr Buford & Thompson, 1987; William L Jr Buford et al., 2012) and Bell-Krotoski (J. Bell-Krotoski, 1991; J. Bell-Krotoski et al., 1993; J. A. Bell-Krotoski & Buford, 1997; J. Bell-Krotoski & Tomancik, 1987) in hand therapy. However, a gap between basic science and clinical practice has historically complicated advancement in this area of knowledge. The book, Evaluation of Sensibility

and Reeducation of Sensation in the Hand (Dellon, 1981) was the first to bridge these two worlds, but unfortunately still today, bringing these worlds together still seems to be a core issue. Moreover, we can see this reflected in clinical practice, where we find the overwhelming majority of sensory and motor testing are still done through traditional manual methods (Dellon, 2016). Traditional methods for sensory testing of pressure, vibration, temperature, and pain are explained in Table 4. These tests can be challenging and difficult to validate and reproduce effectively without inter-evaluator variability.

Table 4. Traditional methods of sensory testing of early loss of protective sensation, vibration, temperature, and pain

What test measures	Test Instrument	Stimulus	Scoring
Early loss of protective sensation	Semmes Weinstein Monofilaments	Different pressure intensity by monofilaments of different diameter.	Patient responds yes/no
Vibration	Tuning fork	Strike tuning fork with force to cause vibration. Apply to affected and nonaffected area.	Patient responds if sensation is the same or different.
	Vibrometer	Apply vibrating head to area to be tested, gradually increase intensity of stimulus.	Patient indicates when he first feels the stimulus.
Temperature	Safety pin	Safety pin is applied at the blunt end at a constant pressure, perpendicular to the patient's skin.	Patient responds sharp or dull after each stimulus.
	Glass test tubes with hot and cold water	Apply cold (4.4ºC) or hot stimuli (46ºC-48ºC) to patient's skin	Patient indicates hot or cold.

3.6.2 Contemporary tools for evaluation: Quantitative sensory testing (QST)

The term sensory reeducation has evolved accompanying advances in the field of neuroscience. Since the turning of the century, Spicher has incorporated a great deal of insight into sensory reeducation by including the principles of neuroplasticity, as a core

part of rehabilitation, transforming sensory reeducation into what he defined in 2006 as somatosensory rehabilitation (Spicher, 2006). Yet still, even Spicher, who has taken sensory testing to a new level, only uses traditional manual methods of evaluation. Theoretically this is most likely due to the great flexibility manual methods offer in comparison with high end automated testing technology, that may not always be designed to be accessible or adaptable to the diversity of conditions patients may have. So, the breach between basic science and clinical application is once more a central issue when evaluating cutaneous sensation. Currently, various computer-assisted sensory testing devices are commercially available, but since the early 1970s the Department of Neurology at the Mayo Clinic, under direction of Peter J. Dyck, have pioneered the concept of QST and developed their own computer-assisted quantitative sensorimotor testing, the CASE IV System (Dyck et al., 1993). The CASE IV Quantitative Sensory Testing System is designed to record and indicate the following parameters during sensory testing:

- Cooling threshold detection
- Vibration threshold detection
- Heat-pain threshold detection, mid-level of pain, and pain tolerance
- Smart Somatotopic sensation testing for touch-pressure threshold measurements

Studies have found the utility of QST for the assessment and monitoring of somatosensory deficits, especially in diabetic and small fiber neuropathies, the evaluation of evoked pains (mechanical and thermal allodynia or hyperalgesia) and for the diagnosis of sensory neuropathies (Backonja et al., 2013; Devigili et al., 2008). QST testing has

also been demonstrated to be important in the prediction and early diagnosis of some sensory neuropathies like diabetic neuropathy (Treede, 2019; Vollert et al., 2017). Furthermore, it has been used in therapeutic trails to assess the effects of certain drugs specifically related to pain (Rolke et al., 2006).

3.7 Previous studies of the somatosensory system in Hepatic Encephalopathy

Studies of sensory processing have shown that both peripheral and central parts of the somatosensory system are compromised in patients with liver cirrhosis and HE (Blauenfeldt et al., 2010; Markus Butz et al., 2013; Chu et al., 1997; May et al., 2014).

Brenner et. al, (2015) found that patients with hepatic encephalopathy have impaired thermal perception, specifically in the detection of cold, which also seemed to parallel with the severity of liver disease. These alterations were observed to mainly emerge in advanced stages of the disease when using the hand as a test site (Brenner et al., 2015).

IV Hypothesis and Aims

IV. Hypothesis and Aims

Mild cognitive impairment has been linked to early changes in the primary somatosensory cortex, which has been proposed as a sensitive marker for cognitive decline. In addition to MHE, due to alterations in the central nervous system, cirrhotic patients may also present alterations in the peripheral nervous system. The study of neurological alterations, sensory perception, motor function, nerve conduction, and autonomic function, could allow the detection of MHE at earlier stages and with greater sensitivity than the current methods used for the detection of MHE.

The aim of this study was to evaluate and characterize the alteration in thermal and mechanical sensitivity and peripheral nerve conduction velocity in cirrhotic patients with and without MHE, their correlation with neurological alterations and the possible contribution of inflammation. To address this general objective, the following specific objectives will be developed:

1. Evaluation of different neurological functions such as attention, concentration, mental processing speed, working memory, and bimanual and visuomotor coordination, using specific psychometric tests. These functions are altered at early stages of MHE.
2. Evaluation of motor functions such as balance, grip strength and motor velocity, processes that all intimately related to cognitive and functional decline.
3. Evaluation and characterization of the alterations in thermal, vibration and Heat Pain sensitivity in cirrhotic patients and healthy controls, and to assess any differences between patients with and without MHE.

4. Sensory and motor nerve conduction studies and autonomic function testing to classify the type of fiber affected (small or large fibers, sensory, motor, or both) and pinpoint the primary pathological process.
5. Correlations of alterations in sensory perception and autonomic function with performance in neuropsychological and motor testing.
6. Evaluation of the contribution of inflammatory parameters to alteration in thermal, vibration and Heat Pain sensitivity associated to MHE.
7. Assessment of diagnostic capacity of alterations in thermal and mechanical sensitivity for detection MHE.

V Methods

V. Methods

Fifty-eight out-patients with liver cirrhosis were recruited from the Clínico and Arnau de Vilanova hospitals in Valencia, Spain. Cirrhosis diagnosis was based on clinical, biochemical, and ultrasonographic data. The exclusion criteria were overt hepatic encephalopathy, recent alcohol intake (<6 months), infection, recent antibiotic use, or gastrointestinal bleeding (<6 weeks), recent use of drugs affecting cognitive function (<6 weeks), presence of hepatocellular carcinoma, and neurological or psychiatric disorders. Participants with insulin-dependent diabetes were also excluded, as they presented more severe polyneuropathies than diabetic patients taking oral antidiabetic drugs (Brenner et al., 2015; Savage et al., 1997). Thirty-nine healthy volunteers without liver disease were also included. Exclusion criteria for all groups were acute or chronic pain and any signs of superficial inflammation or injury in the left foot or hand, to avoid interference with sensitivity results. All participants were included after signing a written informed consent. Study protocols were in accordance with the ethical guidelines of the Declaration of Helsinki and were approved by the Research Ethics Committees of both hospitals. After performing psychometric tests, patients were classified as with or without MHE (see below) and were referred to the neurophysiology unit to undergo electrophysiological and quantitative sensory testing. These tests were performed within the following week after the performance psychometric tests, in order to minimize possible cognitive fluctuations. The composition and characteristics of the groups are given in Table 5.

Table 5. Composition of the different groups and etiology of liver disease

	CONTROL	NMHE PATIENTS	MHE PATIENTS
Number of subjects	39	38	20
Sex (male/female)	13/24	36/2	18/2
Age	64 ± 2	60 ± 1	64 ± 1
Etiology of cirrhosis			
Alcohol	-	18	10
HCV/HBV/HCV + alcohol	-	13/0/1	4/1/0
NASH/NASH + alcohol	-	1/3	4
Other	-	2	1
Diabetes (without/DM NID)	-	31/7	12/8
Child Pugh score (A/B/C)	-	29/9/0	14/6/0
MELD score	-	9.4 ± 2.7	9.1 ± 2.3
† Lactulose	-	3 (8%)	4 (20%)
Beta-blockers	2 (5%)	15 (39%)	5 (25%)
Polyneuropathy (no/yes) (%)	37/2 (95/5)	24/14 (63/37)	12/8 (60/40)

† Values are expressed as mean ± SD. MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively; HBV: hepatitis B virus; HCV: hepatitis C virus; NASH: non-alcoholic steatohepatitis; DM NID: diabetes mellitus without insulin dependence; MELD: model end-stage liver disease

5.1. Neuropsychological Testing

5.1.1 Psychometric Hepatic Encephalopathy Score

The Psychometric Hepatic Encephalopathy Score (PHES), considered as the golden standard tool to detect MHE, consists of a battery of psychometric tests (Ferenci et al., 2002; Weissenborn, 2015; Rudler et al., 2021).

It is composed of five tests:

1- **Digit symbol test (DST)**, assesses processing speed and working memory (Figure 6A). It consists of a series of numbers (1-9) each of which corresponds to a different symbol. Subjects are asked to insert symbols in the blank squares below the numbers using key provided. The score is calculated by the sum of correct number to symbol pairings made in 90 seconds.

2- Number connection test-A (NCT-A) evaluates attention and processing speed (Figure 6B). In this test the subject must draw a line, without separating paper and pencil, between a series of disorganised numbers (1-25), starting with the smallest and ending with the largest. The time taken to complete the task is used to obtain the final score.

3- Number connection test-B (NCT-B) also evaluates attention and processing speed (Figure 6C). The subject has to connect the numbers (1-13), in ascending order, while ordering letters (A-L), in alphabetical order which must be united alternately (1-A-2-B-3-C…). The time taken to complete the task is recorded.

4- Serial dotting test (SDT) assesses visuospatial coordination (Figure 6D). In this test the subject must dot the middle of every circle, as fast as possible. The time taken to complete the task is recorded.

5- Line tracing test (LTT), like SDT, examines visuospatial coordination (Figure 6E). Subjects are asked to trace a line between the two guidelines as quickly and accurately as possible without separating paper and pencil and without moving the paper. The time taken to complete the task and the number of errors made are recorded.

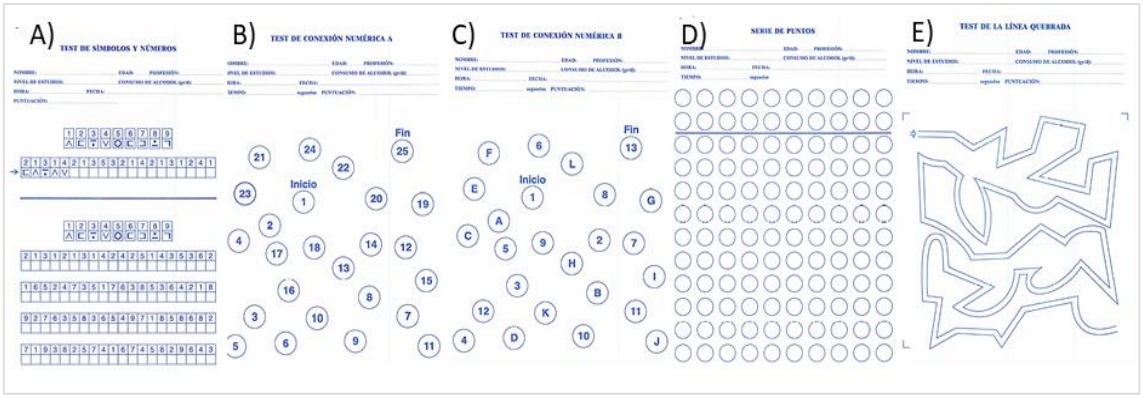

Figure 6. Psychometric Hepatic Encephalopathy Score (PHES). Assess attention, visual perception, and visuocoordination abilities. Digit Symbol Test (A): subjects are asked to insert symbols in the blank squares below the numbers using the key provided. Number Connection Tests A (B) and B (C). Serial Dotting (D). Line tracing (E).

The global PHES score was calculated and adjusted for both age and level of education by Spanish normality tables (http://www.redeh.org/TEST_phes.htm). Patients were considered as having MHE when the score was equal or below −4 points (Weissenborn, 2015).

5.1.2 STROOP Test

Stroop test (Figure 7) was used to evaluate attention, cognitive flexibility and resistance to interference (Stroop, 1935) . The test consists of three parts. The first is the congruent task, in which the subject is presented a list of colour names printed in black font (Figure 7A). The subject is asked to read as many words as possible in 45 seconds. The second task is the neutral task (Figure 7B), the subject here is presented with a list of circles of different colours. Here the participant is asked to name as many circle colours as possible in 45 seconds. The last task, called incongruent (Figure 7C), is a list of name colours (green, blue, yellow and red), in which the words are in a different coloured font from that of the written colour name. The subject must indicate the colour of the font, not read the words, as many as possible in 45 seconds. In all tasks, when the subject reaches

the end of list before the time is up, they start from the top of the list again. Three direct results were obtained (maximum number of items in each part), which were corrected in relation to age and from which the interference parameter was calculated. The obtained values were interpolated through normative data of the Spanish population (Golden, 2001).

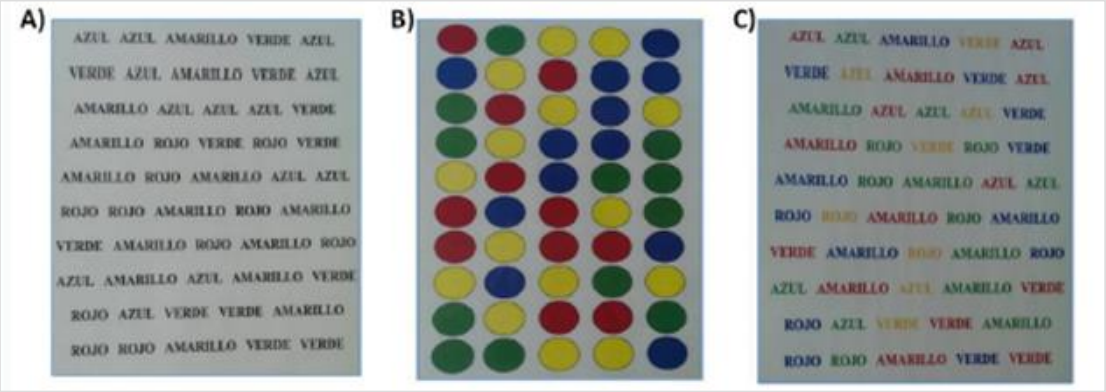

Figure 7. Stroop Test. Used to evaluate attention, cognitive flexibility, and resistance to interference A) **C**ongruent task B) Neutral task C) Incongruent task (Stroop, 1935)

5.1.3 D2 Test

D2 Test (Figure 8) assesses concentration, selective and sustained attention through the localization of the letter "d" accompanied by two apostrophes (Brickenkamp, 2009), not always placed in the same location, in a line of letters (d or p, with one or two apostrophes). The objective is to locate as many d with two apostrophes in the line, in 20 seconds and then continue to the next line until no more are left. There are 14 lines with 47 elements corresponding to visual stimuli. The evaluation of the test is done through a series of parameters:

- Total responses (TR) are the corresponding box number lastly marked of each line. The sum of the total responses of the 14 lines provides a measurement of processing speed and the amount of work done.
- Total of correct answers (TA) is the number of correct items that have been marked. The sum of responses of the 14 lines measures processing accuracy.
- Omissions is the number of errors by omission, therefore the number of correct elements not marked until the last marked element. The omissions of the 14 lines are recorded.
- Errors are the number of incorrect items marked; these are counted until the last marked item. The sum of errors of the 14 lines reflects the capacity of processing accuracy and inhibitory control.
- Total effectivity (TOT) of the test is calculated by the difference of the total responses by the sum of errors and omissions of the 14 lines.
- Index of concentration (CON) is calculated as the difference of the total of correct answers and the number of errors.

All calculated scores were transposed for percentile scoring through normative data table equivalents (Brickenkamp, 2009; Giménez-Garzó et al., 2017).

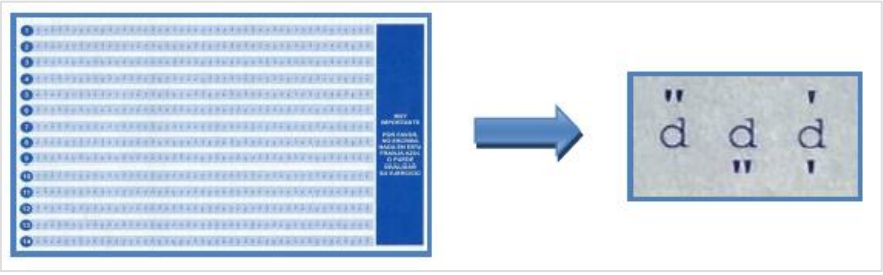

Figure 8. D2 Test. Assesses concentration, selective and sustained attention through the localization of the letter "d" accompanied by two apostrophes (Brickenkamp, 2009).

5.1.4 Digit Span Test

The Digit Span Test (DST) evaluates immediate and working memory (Wechsler Adult Intelligence Scale, WAIS) (Wechsler, 1955). It consists of two parts: 'digits forward' (Figure 9A), and 'digits backward' (Figure 9B). In digits forward the subject must repeat each number sequence, ranging from three to nine elements, in the same order as it was given. Starting with the sequence compromised of less elements and increasing the number of elements when the sequence was repeated correctly (two trials were allowed for each sequence). The test is continued until two consecutive sequences are incorrect or all sequences are correctly repeated. In digits backward, the number sequences are ranged from 2 to 8 elements. After hearing the sequence, the subject must say the sequence in reverse order. The test is continued with the same criteria as digits forward (Curran et al., 2004).

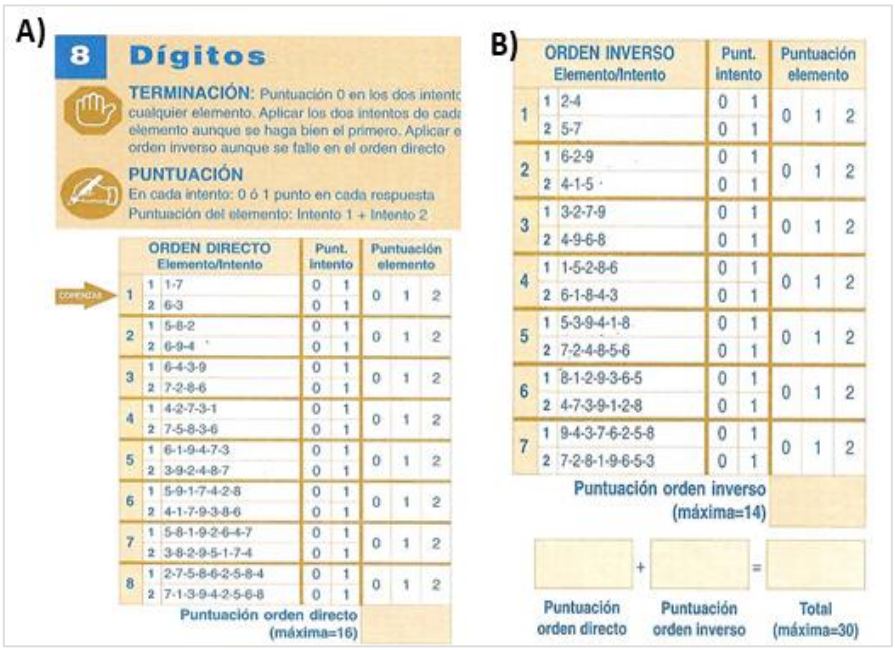

Figure 9. Digit Span Test (DST). This test valuates immediate and working memory. A) Digits forward B) Digits backward

5.1.5 Oral Symbol Digit Modalities Test

The Oral Symbol Digit Modalities Test (Oral SDMT) measures processing speed and selective attention (Ryan et al., 2020). The test consists of showing the subject symbols associated with numbers then the subject must, verbally, relate in 90 seconds as many symbols as possible with the correct corresponding numbers (Smith, 1968). The score is the number of correct pairings (Giménez-Garzó et al., 2017).

5.1.6 Letter-Number Sequencing Test

The Letter-Number Sequencing test measures working memory (Egeland, 2015). It consists of three-series blocks containing mixed letters and numbers (Figure 10). The number of elements increases as the test progresses. After hearing a series, the subject must sort the items by saying the numbers in ascending order and then the letters arranged alphabetically. The test continues until the subject fails three series. The total correct answers are registered.

	Intento	Elemento	Respuesta correcta	Punt. intento		Puntuación elemento			
1	1	L-2	2-L	0	1				
	2	6-P	6-P	0	1	0	1	2	3
	3	B-5	5-B	0	1				
2	1	F-7-L	7-F-L	0	1				
	2	R-4-D	4-D-R	0	1	0	1	2	3
	3	H-1-8	1-8-H	0	1				
3	1	T-9-A-3	3-9-A-T	0	1				
	2	V-1-J-5	1-5-J-V	0	1	0	1	2	3
	3	7-N-4-L	4-7-L-N	0	1				
4	1	8-D-6-G-1	1-6-8-D-G	0	1				
	2	K-2-C-7-S	2-7-C-K-S	0	1	0	1	2	3
	3	5-P-3-Y-9	3-5-9-P-Y	0	1				
5	1	M-4-E-7-Q-2	2-4-7-E-M-Q	0	1				
	2	W-8-H-5-F-3	3-5-8-F-H-W	0	1	0	1	2	3
	3	6-G-9-A-2-S	2-6-9-A-G-S	0	1				
6	1	R-3-B-4-Z-1-C	1-3-4-B-C-R-Z	0	1				
	2	5-T-9-J-2-X-7	2-5-7-9-J-T-X	0	1	0	1	2	3
	3	E-1-H-8-R-4-D	1-4-8-D-E-H-R	0	1				
7	1	5-H-9-S-2-N-6-A	2-5-6-9-A-H-N-S	0	1				
	2	D-1-R-9-B-4-K-3	1-3-4-9-B-D-K-R	0	1	0	1	2	3
	3	7-M-2-T-6-F-1-Z	1-2-6-7-F-M-T-Z	0	1				
				Puntuación directa (máxima=21)					

Figure 10. Letter-Number Sequencing test. Assesses working memory.

5.1.7 Coordination testing

Two coordination tests were used:

- Bimanual coordination (Figure 11A): the subject was presented with a pegboard test where they were asked to transfer all the pins, in the order indicated (up- down and out-in or down- up and out-in), while using both hands at the same time. The time taken to complete the test was measured and the test was repeated twice in each direction. The test involves gross movements of upper extremities and fine motor dexterity.
- Visuomotor coordination (Figure 11B): This test involves hand-eye coordination and visuo-construction. It consists of an open box, on one side there are metallic prisms, which are all the same, and on the other side a metal plate with openings where to fit the prisms but in each opening has a different orientation. Using the dominant hand, the subject must place the prisms in order, left to right, in the slots of the plate, the test is done twice and time was measured (Felipo et al., 2012).

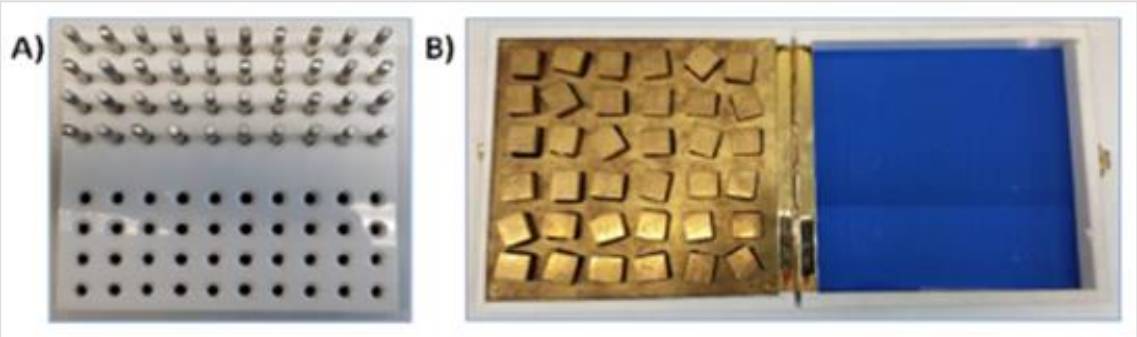

Figure 11. Coordination tests. A) Bimanual coordination test. B) Visuomotor coordination test.

5.2 Biomechanical parameters

Balance, gait and hand function were assesed through the following methods:

- Balance: Functional postural balance was assessed on a single force plate by the NedSVE®/IBV system (vs. 5.1.0, 2013) (Instituto de Biomecánica de Valencia, Valencia, España). Balance control measurement included Rhythmic Weight Shifting tests (RWS), which assessed the ability to rhythmically move the centre of pressure (COP) in the anteroposterior (AP- RWS) and mediolateral (ML- RWS) planes, between two targets located at 60% of the subject's stability limits, this computerized version of modified clinical testing of sensory interaction and balance (mCTSIB), consisted of three 30 second Romberg trials under four sensory conditions: eyes open (REO) or closed (REC) on a firm surface, and eyes open (RFEO) or closed (RFEC) on unstable (foam) surface. A mean global score was calculated. Finally, a score of 100% reflected normality and other scores reflect discrepancies from an age and height matched normative data provided from the system (Urios et al., 2017).
- Gait assessment. Gait evaluation was carried out with two photocells and two force platforms (Dinascan Biomechanical Institute/IBV of Valencia, Valencia, Spain) and NedAMH/IBV software (version 5.1.0, 2013, Biomechanical Institute of Valencia, Valencia, Spain). Participants walked barefoot along a 10m long corridor at a comfortable self-selected speed. The force platforms were located at the center of the corridor to record the central passage and to prevent acceleration and deceleration at the beginning and end of the walking cycle. The recorded result of the walking task was the overall assessment (%) and the walking speed (m/s).

- Hand strength: Lower grip strength is a marker of sarcopenia, age-related loss of skeletal muscle mass and function. In this study an isometric electronic dynamometer (application Ned Discapacidad/IBV with specific hand modulator instrument, Ned-VEP/IBV) was used to measure the strength of both the left and right grip and lateral and distal pinch strength. The result of maximum contraction was obtained from the mean of three strength trials.
- Finger tapping test consisted of a button press speed task, with three trials for each index fingers (right and left) each trial was independently measured. The mean of the three trials was used as the total result of presses per minute for each index finger.

5.3 Biochemical parameters

Blood extraction and psychometric testing were performed on the same day in order to minimise variability.

5.3.1 Ammonia level

Ammonia levels were measured immediately after blood extraction for analysis by means of the Ammonia Checker II® (Arkray Factory, Inc.) (Figure 12), a device based on microdiffusion that quantifies ammonia blood levels from a blood sample of 20 μL. The Ammonia Checker reaction kit consists of strips that contain a receiving layer sample, composed of boric acid (42.6 mg) and sodium hydroxide (18.7 mg) and an indicator layer, composed of green bromocresol (4.0 mg). When the blood sample is applied to the receiving layer of the strip, the impregnated pH buffer agent (borate buffer)

dissolves making the sample alkaline. The ammonium ions (NH4+) of the sample pass, through the alkaline pH, to ammonium gas (NH3) that continues through to the pores of the spacer to the indicator layer (green bromocresol) and produces color. The degree of green coloration is proportional to the concentration of ammonium gas produced, so the Ammonia Checker device can then quantitatively determine the level of ammonium in the sample by measuring the color of the strip. The ammonia level appears on the screen in μmol/L. The range of detection of this method is 7-286 μmol/L. Levels under 54 μmol/L are considered to be normal.

Figure 12. Ammonia Checker II® (Arkray Factory, Inc.)

5.3.2 Proinflammatory interleukin 6

Proinflammatory interleukin 6 (IL-6) from serum was quantified by ELISA kit (Thermo Scientific, USA) with a detection limit of 1pg/mL. First, serum samples were extracted with 5 mL BD vacutainer tubes without ethylenediaminetetraacetic acid and centrifuged at 3000 x g for10 minutes at room temperature. The serum was collected and stored at -80 °C in aliquots of various volumes. These aliquots were later unfrozen in order to measure the parameter of interest in this case IL-6. The protocol used followed the commercial kit (Human IL-6 Platinum ELISA Affymetrix eBioscience ref. BMS213/2) indications. A summary of the kit protocol is detailed below:

1. Determine the number of microwell strips required.

2. Wash microwell strips twice with Wash Buffer.

3. Standard dilution on the microwell plate: Add 100 µL Assay Buffer (1x), in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.

4. Add 100 µL Assay Buffer (1x), in duplicate, to the blank wells.

5. Add 50 µL Assay Buffer (1x) to sample wells.

6. Add 50 µL sample in duplicate, to designated sample wells.

7. Prepare Biotin-Conjugate. 8. Add 50 µL Biotin-Conjugate to all wells.

9. Cover microwell strips and incubate 2 hours at room temperature (18–25°C).

10. Prepare Streptavidin-HRP.

11. Empty and wash microwell strips 4 times with Wash Buffer.

12. Add 100 µL diluted Streptavidin-HRP to all wells.

13. Cover microwell strips and incubate 1 hours at room temperature (18–25°C).

14. Empty and wash microwell strips 4 times with Wash Buffer.

15. Add 100 µL of TMB Substrate Solution to all wells.

16. Incubate the microwell strips for about 10 minutes at room temperature (18–25°C).

17. Add 100 µL Stop Solution to all wells.

18. Measure color intensity at 450 nm with a microplate reader (Multiskan Ascent).

The results are then calculated by interpolating the absorbance obtained in the samples to the standards pattern curve. The concentration of cytokine present is expressed as pg/µl.

5.4 Neurophysiological Studies

All neurophysiological parameters were studied on the same day, including conduction study, autonomic function study and quantitative sensory testing (QST). Neurophysiological studies allowed the detection of polyneuropathy (PNP), the investigation of the type and progression of PNP, and gave insight into the relationship of PNP with the disease and the possible origin of later evaluated sensory deficits. Each laboratory must elaborate their own independent protocol of neurophysiological evaluation due to the differences that exist between testing equipment, techniques, and individual characteristics of the study population.

5.4.1 Neurophysiological Studies of Large Calibre Fibres: Nerve Conduction Study

The nerve conduction study (NCS) evaluated Sensory and Motor Nerve Conduction with Synergy, version 22.0.0.144 and components: UltraPro S100 version 1 and UltraProS100 DSP version 591. Motor NCS was done on the following nerves: right cubital, right tibial and right peroneal, and the left tibial. Sensory NCS was carried out on the peroneal (right and left), cubital and radial (left), and sural (right) nerves. Parameters measured were amplitude, latency, and conduction velocity. Latency measures in milliseconds (ms) the nerve conduction time from which stimuli begins to the moment of the evoked response. Amplitude is the median value, in millivolts (mV), of the negative peak and positive peak of the evoked response and it assesses the number of stimulated axons. Finally, conduction velocity, expressed in m/s, is calculated by measuring the length between two stimulated points of the same nerve and dividing it by the time which is the difference between proximal latency and distal latency. The data obtained from the

NCS was used to detect large nerve fibre damage, polyneuropathy. The conduction study protocol used for the diagnosis of polyneuropathy was based on those described by Falck and Stålberg (1997) and Preston and Shapiro (2012).

5.4.2 Neurophysiological Studies of Small Calibre Fibres: Quantitative Sensory Testing (QST) and Autonomic Testing

5.4.2.1 Quantitative Sensory Testing (QST)

Quantitative Sensory Testing (QST) was evaluated by the CASE IV System WR Testworks (Figure 13A). The modalities of sensation monitored were; the Vibratory Detection Threshold (VDT) that evaluates large diameter sensory myelinated fibres, A alpha; Cooling Detection Threshold (CDT) that allows measurement of mainly small diameter myelinated fibres, A delta; and Heat Pain Detection Threshold (HPDT) which evaluates unmyelinated C fibres.

The calculation of these sensory thresholds was done by administering a series of non-invasive vibratory or thermal stimuli, corresponding to a set of 25 standardized vibratory and thermal stimulation levels, according to a one-time-period 4, 2, 1 Stepping Algorithm. This algorithm determines how the stimuli are presented. During a given test, subsequent stimuli may be dependent on a patient's response. These tests have a total of 20 stimulus trials; each trial corresponds to one time period. During this time period, the stimulus may or may not be delivered (five periods of null stimuli are placed randomly to prevent false results). The green light will flash on the Patient Cue Device, signalling

the beginning of a trial. Then, a “1” will be presented, signalling the time period (Figure 13D). The subject must try to determine whether a stimulus (vibration or thermal) was delivered. The patient then answers by pressing “yes” or “no” on the Patient Response Device (Figure 13E) for cooling and vibration tests. For heat-pain testing the subject answers a number from 0 to 10, 0 being no pain and 10 being maximum pain possible.

The VDT stimuli is administered by the Vibration Stimulator (Figure 13B) consisting of an electronic actuator, a galvanometer, set at 125 cycles per second, variable between 0 and 350 micrometres. The CDT and HPDT tests are done by the thermal Stimulator (Figure 13C), a ceramic plate, held in place by a Velcro strap, which produces a specified temperature, which can be varied from 8.0 to 50.0 degrees C, with accuracy of 1.25 to 0.25 degrees C, on a 9.0-square-centimeter stimulating surface (traceable to National Institute of Standards and Technology, NIST, standards).

Prior to testing, the Thermal Stimulator adjusts itself to match the patient’s baseline skin temperature. For statistical normalization purposes, the baseline temperature was of 30ºC for Cooling test and 34 ºC for Heat-Pain test. For high-magnitude thermal (warming) stimuli, a holding time is added to the waveform so that the absolute temperature is typically limited to 50 degrees C. The plateau lengthens the time that the stimulus is administered, providing more heat over time, ensuring the same physiologic sensation as a higher pyramidal-shaped waveform. For high-magnitude thermal (cooling) stimuli, the absolute temperature is limited to 8 degrees C.

A total of six tests were performed, 3 modalities VDT, CDT and HPDT, on two test sites, the hand (Figure 13, 2 for VDT and Figure 13, 4 for CDT and HPDT), and the foot (Figure 13, 1 for VDT and Figure 13, 4 for CDT and HPDT), all in this order.

Results were represented by the sensory thresholds of each individual test in Just Noticeable Differences (JND), corresponding to the mean of the level of stimuli just detectable by the subject during the test. Also, from the data recollected from the control group a normal range was calculated and the quantity of tests which were out of the normal range were summed up for each individual patient, considering: total of irregular hand tests, total of irregular foot tests and the total of tests out of normal range. Finally, the total time to complete each test was also considered and measured in seconds.

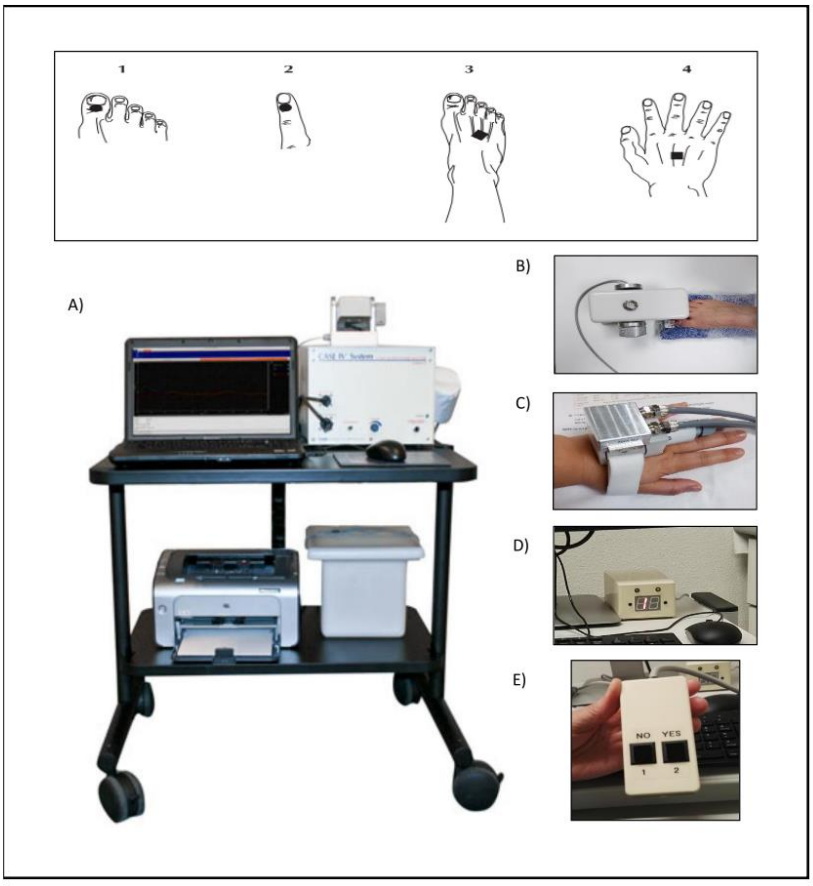

Figure 13. Quantitative Sensory Testing components. The CASE IV system (A) consists of: The Vibration Stimulator (B), a galvanometer, set at 125 cycles per second, variable between 0 and 350 micrometres. The thermal Stimulator (C), a ceramic plate, held in place by a Velcro strap, which produces a specified temperature, which can be varied from 8.0 to 50.0 degrees C. These stimulus apparatuses are placed either on the hand or foot, 1 and 2 being for Vibration Detection tests and 3 and 4 being for thermal tests. The subject is then asked to pay attention to the box (D), when the light turns on, they must be prepared to attend to the stimulus which is administered or not when the one appears. When the number turns off, the subject must respond if a stimulus was administered yes or no on the remote device (E).

5.4.2.2. Autonomic Testing

The **sympathetic skin response (SSR)** explored the autonomic nervous system by means of changes in skin potential. The sources of these potentials are sweat glands and epidermis, related to sudomotor response and therefore the thermoregulation system (Murota, 2016). When the autonomic nervous system is affected by SFN, SSR latency and, or amplitude will be delayed (Raasing et al., 2021). SSR, like the nerve conduction study, was conducted by the Synergy UltraPro S100. A registering electrode was placed on the right palm with a reference electrode on the back of the hand. The electrodes used were common to the EMG technique, with a filter of low frequencies of 0.5 Hz. The stimulus used was a small electric stimulation and the parameters correspond to NCS. The amplitude of SSR was considered abnormal when it was lower than 1.1 mV. This threshold was calculated from the mean of amplitude of sympathetic skin response of controls minus 2 standard deviations.

Heart rate variability (**HRV**) was evaluated to detect alteration in the cardiovascular autonomic nervous system. Resting heart rate via the vagal tone, and heart rate variability, which consisted of variation of the RR interval of the electrocardiogram, was registered by evaluating fluctuations in heart rate. Duration of RR intervals recorded over time reflect the influence of both sympathetic and parasympathetic nervous systems in heart rate modulation. Among the different activation manoeuvres, Valsalva manoeuvre, hyperventilation, and orthostatic tests (from lying to sitting position) were tested (Chémali & Chelimsky, 2014). Registering electrodes were placed on both wrists above the radial artery.

5.5 Statistical analysis

Values are given as mean ± standard error of mean (SEM), unless otherwise specified. D'Agostino and Pearson omnibus normality test was used to test variable normality. Between-group differences were analysed using one-way ANOVA followed by post-hoc Tukey's multiple comparisons test. For non-parametric variables, the Kruskal-Wallis test was performed, followed by Dunn's multiple comparisons test. Results were analysed by GraphPad PRISM Version 7. Bimanual and visual-motor coordination tests were analysed using univariate analysis of covariance (ANCOVA) with age included as covariate, followed by post-hoc Bonferroni. Analyses of contingency tables were performed by Fisher's exact test. We evaluated the predictive capacity of QST parameters for MHE using ROC (receiver operating characteristic) curves. Pearson correlation analysis and ROC analyses were performed using the SPSS software, Version 20 (SPSS Inc, Chicago, IL, USA) The probability level accepted for significance was $p < 0.05$.

VI Results

VI. Results

6.1 Classification of patients with PHES score

PHES scoring results (Figure 14) allowed the classification of cirrhotic patients into 38 patients without MHE (NMHE) and 20 with MHE.

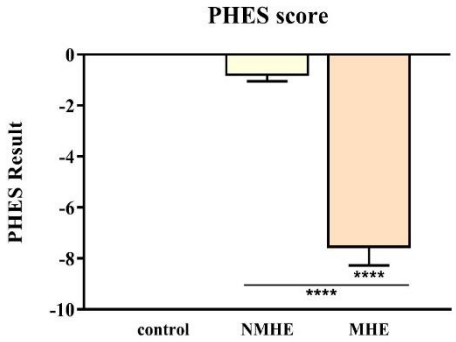

Figure 14. PHES score results. Cirrhotic patients with minimal hepatic encephalopathy show low PHES scoring. Significantly different results were found between cirrhotic patients with and without MHE and between MHE patients and healthy controls. Results are mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; MHE, NMHE: patients without and with minimal hepatic encephalopathy, respectively.

PHES scoring for each group are displayed in Table 6. MHE patients obtained significantly different values compared to controls ($p < 0.0001$) and NMHE patients ($p < 0.0001$). No differences were found between NMHE patients and healthy controls.

6.2 Neuropsychological testing

Further psychometric tests were conducted to evaluate selective and sustained attention, processing speed and coordination (Table 6). Significant differences between MHE patients and controls are found in all tests. Differences between MHE and NMHE patients are described below:

- *Bimanual coordination*: Cirrhotic patients with MHE took significantly more time (3.4 ± 0.3 min) ($p<0.001$) than patients without MHE (2.3 ± 0.1 min) to complete the task. No significant differences were found between NMHE and controls.
- *Visuomotor coordination*: Like the previous test, MHE patients took significantly more time (3.7 ± 0.2 min) ($p<0.001$) than cirrhotic patients without MHE (2.9 ± 0.1 min). No significant differences were found between NMHE and controls.
- *D2 Test*: Various scoring measures were compared. In all scoring (TR, TOT, CON and TA) patients had significantly lower results than controls, being lower for NMHE patients and even lower still for MHE patients. Significant differences were found between MHE patients and NMHE patients in TR ($p=0.03$), TOT ($p=0.05$) and CON ($p=0.04$) scoring but no significant differences were found between patient groups in TA values (Table 6).
- *Stroop test*: Congruent task was not significantly different between controls and NMHE patients, although it was between MHE patients and controls ($p<0.01$). Furthermore, between MHE and NMHE patients there was a significant difference ($p=0.04$). Neutral task scoring showed an even bigger difference between MHE patients and NMHE patients ($p=0.001$). Both groups of patients scored significantly less compared to controls. Incongruent task showed similar findings to neutral task in which NMHE patients scored significantly less than controls and MHE patients even less. Significant differences were found between MHE patients and NMHE in this task ($p=0.02$) (Table 6).

- *Oral SDMT*: Both patient groups scored significantly less than healthy subjects. Patients with MHE performed less correct pairings than cirrhotic patients without MHE ($p<0.001$).
- *Digit Span test*: This test showed no significant differences between patient groups nor between NMHE and controls. However, MHE patients did score significantly less that healthy subjects.
- *Letter-Number Sequencing test*: Results from this test showed a significant difference between NMHE and MHE patients ($p=0.02$) although no significant differences between NMHE patients and controls were found. MHE patients did score significantly worse than healthy subjects.

Table 6. Neuropsychological testing

Test	Controls	NMHE p vs. Control	MHE p vs. Control	MHE Patients p vs. NMHE	Global ANOVA p Values
PHES score	0.0± 0.2	-0.8± 0.2	-7.5 ± 0.7***	<0.001	<0.001
Bimanual coordination (min)	2.2± 0.1	2.3 ± 0.1	3.4 ± 0.3 ***	<0.001	<0.001
Visuomotor coordination (min)	2.7 ± 0.1	2.9 ± 0.1	3.7 ± 0.2 **	<0.001	<0.001
d2 Test					
TR Values	378 ± 19	322 ± 11 *	271 ± 14 ***	0.03	<0.001
TOT Values	366 ± 16	292 ± 12 **	245 ± 15 ***	0.05	<0.001
CON Values	146 ± 7	114 ± 6 *	89 ± 10 ***	0.04	<0.001
TA Values	142 ± 7	117 ± 6 *	98 ± 7 **	ns	0.001
Stroop Test					
Congruent Task †	103 ± 4	94 ± 3	81 ± 4 **	0.04	0.003
Neutral Task †	78 ± 3	69 ± 2 *	57 ± 2 ***	0.001	<0.001
Incongruent Task †	47 ± 2	37 ± 2 **	30 ± 2 ***	0.02	<0.001
Oral SDMT (correct pairings)	44 ± 2	38 ± 1 *	26 ± 2 ***	<0.001	<0.001
Digits Span-Total score	14 ± 0.7	12 ± 0.6	11 ± 0.7 **	ns	0.007
Letter-Number Sequencing test (right answers)	9 ± 0.6	7 ± 0.6	5 ± 0.7 **	0.02	0.001

Values are expressed as mean ± SEM. Differences between groups were analysed using one-way ANOVA followed by post-hoc Tukey. For bimanual and visual-motor coordination tests, univariate analysis of covariance (ANCOVA) was performed, with age included as a covariate, followed by post-hoc Bonferroni. Significant differences compared to controls are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. MHE and NMHE: patients with and without Minimal Hepatic Encephalopathy: respectively; PHES: Psychometric Hepatic Encephalopathy Score; d2 test: TR: Total number of characters processed; TOT: Total correctly processed; CON: Concentration performance; TA: Total right answers. † Stroop test: Congruent task: number of words read in 45 s; Neutral task: number of colours read in 45 s; Incongruent task: number of items completed in 45 s. Oral SDMT: Symbol digit modalities test (oral version).

6.3 Biomechanical parameters

Biomechanics results are represented in Figure 15. Balance results (Figure15A) and finger tapping speed test (Figure15 B) indicated a significant difference between NMHE and MHE patients ($p<0.05$). MHE patients showed lower gait velocity ($p<0.01$) and worse global gait result ($p<0.05$) than patients without MHE (Figure 15C, D). Grip strength (Figure 15E) showed no significant differences between patients nor did pinch strength (Figure 15F) except for left lateral finger which did show significant differences between NMHE and MHE ($p<0.05$).

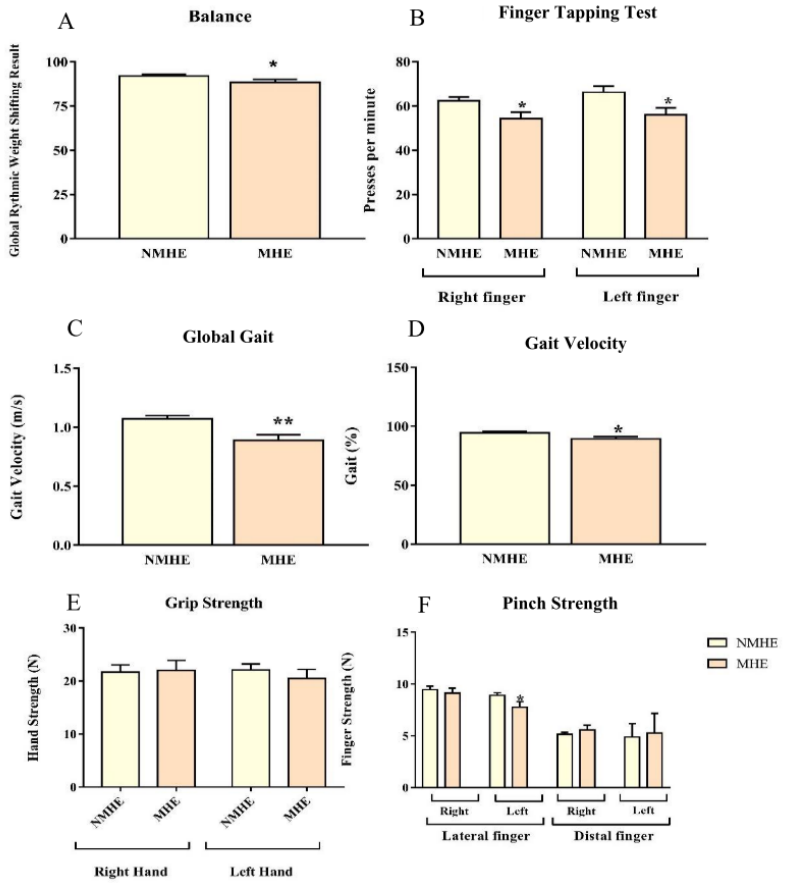

Figure 15. Biomechanics results. (A) Balance; (B) Finger Tapping Test; (C) Global Gait Result (%); (D) Gait velocity; (E) Grip strength; (F) Pinch strength. MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively. Results are mean ± SEM. * $p < 0.05$; ** $p < 0.01$

6.4 Biochemical parameters

Blood ammonium levels are significantly increased in both groups of patients compared to controls (Figure 16, Table 7). Serum IL-6 levels (Figure 16) are significantly increased in patients with MHE compared to NMHE patients ($p<0.01$) and to controls ($p<0.0001$). Patients without MHE showed significantly higher serum IL-6 levels than controls ($p<0.001$) (Figure 16, Table 7).

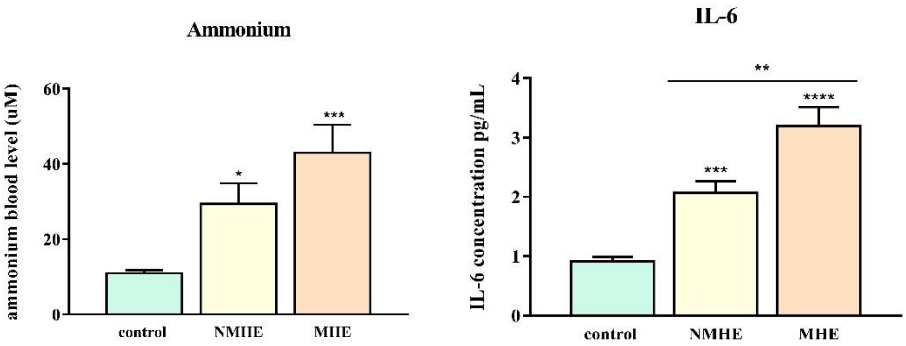

Figure 16. **Patients with MHE have increased levels of ammonium and IL-6 in blood compared to healthy control subjects and NMHE patients**. Between-group differences were analysed using Kruskal-Wallis test followed by Dunn's multiple comparisons test. Group differences are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. MHE, NMHE: patients without and with minimal hepatic encephalopathy, respectively.

Table 7. Biochemical results

	Controls	NMHE p vs. Control	MHE p vs. Control	MHE patients p vs. NMHE
AMMONIUM	11 ± 0.83	29.4 ± 5.5*	43 ± 7.5***	0.0670
IL-6	0.91 ± 0.07	2.1 ± 0.2***	3.2 ± 0.3****	$p < 0.01$

Ammonium level is in micromole per litre. IL-6: interleukin 6 is expressed in picograms per millilitre. Between-group differences were analysed using Kruskal-Wallis test followed by Dunn's multiple comparisons test for non-parametric values. Differences with control group are indicated by asterisks: * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$. MHE, NMHE: patients without and with minimal hepatic encephalopathy, respectively.

6.4 Study of large calibre fibres

Nerve conduction study is shown in Figure 17. Sensory nerve conduction study results demonstrate reduced amplitude in cirrhotic patients in all nerves evaluated (Figure 17A), whereas latency was increased in ulnar and radial nerves (Figure 17B). Conduction velocity of sensory nerves was reduced in ulnar and radial nerves in patients (Figure 17C). Significant differences were found between patients with and without MHE (Figure 17B) in latency of the sural nerve ($p<0.05$).

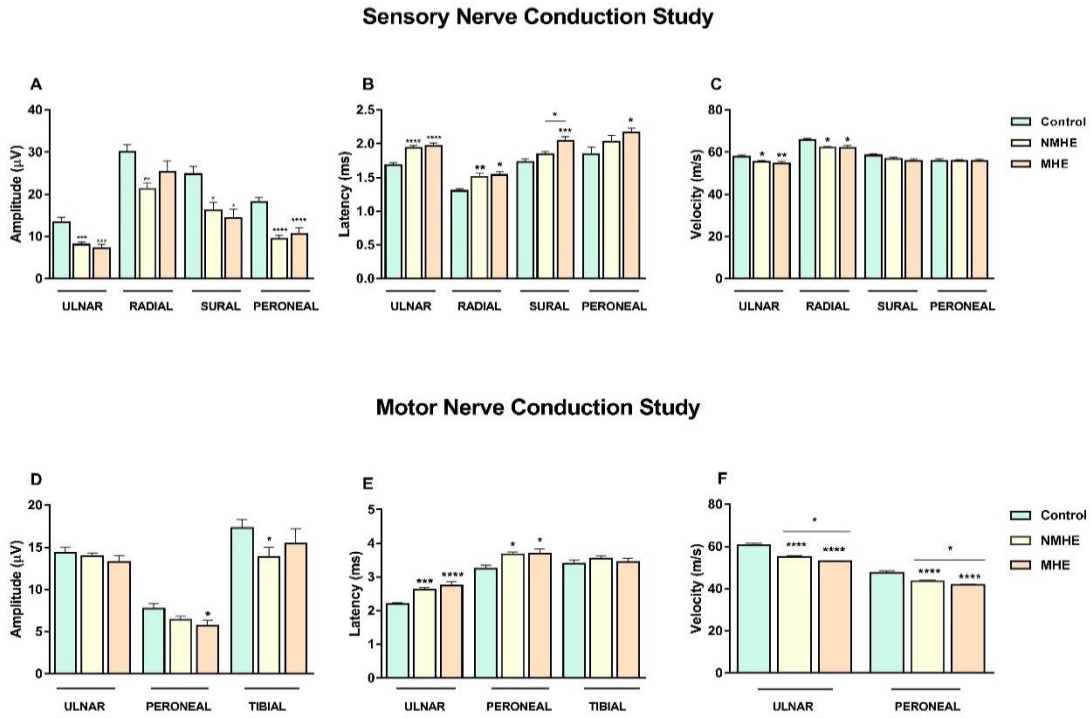

Figure 17. Nerve conduction study. Cirrhotic patients with and without MHE show alterations in sensory and motor nerve conduction. (A–C): Sensory-nerve conduction. (A) Amplitude; (B) Latency; (C) Nerve conduction velocity. Data are for these sensory nerves: ulnar, radial superficial, sural, and superficial peroneal nerves. (D–F): Motor-nerve conduction. (D) Amplitude; (E) Latency; (F) Nerve conduction velocity. Data for ulnar, peroneal, and tibial motor nerves are shown. MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively. Results are mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Motor nerve conduction study show reduced amplitude in peroneal nerve of MHE patients compared to controls and tibial nerve of NMHE patients compared to controls (Figure 16D). Latency results indicate an increased delay in both group patients in both ulnar and peroneal nerves (Figure 16E). Conduction velocity was diminished in patient groups in comparison with controls (Figure 16F) and demonstrated significant differences between NMHE and MHE patients in ulnar and peroneal nerves ($p<0.05$).

6.5. Study of small calibre fibres

Study of small calibre fibres was conducted by QST, SSR and HRV testing (Table 8).

Table 8. QST and autonomic testing results

QST Parameters		Controls	NMHE Patients p vs Control	MHE Patients p vs Control	MHE Patients p vs. NMHE	ANOVA Global p Values
Vibration	hand	7 ± 0.5	9 ± 0.5**	10 ± 0.5 **	ns	<0.0001
Detection (JND)	foot	13 ± 0.6	7 ± 0.4****	17 ± 0.6****	ns	<0.0001
Cooling	hand	7 ± 0.3	10 ± 0.5****	11 ± 0.8 ****	ns	<0.0001
Detection (JND)	foot	8 ± 0.4	13 ± 0.7****	16 ± 1 ****	0.04	<0.0001
Heat Pain 0.5	hand	16 ± 0.5	18 ± 0.5**	19 ± 0.7 **	ns	0.0006
(JND)	foot	18 ± 0.4	19 ± 0.3**	21 ± 0.5 ****	0.01	<0.0001
Heat Pain 5.0	hand	20 ± 0.5	22 ± 0.4**	22 ± 0.7 *	ns	0.004
(JND)	foot	21 ± 0.3	22 ± 0.2	23 ± 0.6 **	0.04	0.005
Vibration	hand	127 ± 1	132 ± 2 *	138 ± 3 **	ns	0.004
Detection time (s)	foot†	127 ± 2	132 ± 2	141 ± 4 ***	0.04	0.0007
Cooling	hand†	141 ± 1	154 ± 5	155 ± 3 **	ns	0.004
Detection time (s)	foot†	144 ± 2	176 ± 8 **	213 ± 15 ****	0.01	<0.0001
Heat Pain time (s)	hand	115 ± 8	182 ± 11 ***	189 ± 18 ***	ns	0.0001
	foot	125 ± 8	149 ± 9	187 ± 14 ***	0.01	0.0009
Autonomic testing						
R-R Interval	Basal†	3.2 ± 0.7	3.1 ± 0.5	2.0 ± 0.2	ns	0.264
variation (%)	Hyperventilation	11.4 ± 2.0	5.9 ± 0.8 *	7.1 ± 1.8	ns	0.021
	Valsalva	15.6 ± 2.1	9.3 ± 1.3	9.9 ± 2.0	ns	0.389
	Orthostatic test	8.2 ± 2.5	5.5 ± 1.0	3.5 ± 0.7	ns	0.130
Sympathetic skin	Amplitude	4.1 ± 0.6	2.6 ± 0.3	1.5 ± 0.3 **	0.047	<0.001
response	Latency	1.40 ± 0.04	1.57 ± 0.04 **	1.59 ± 0.06 *	ns	0.015

Values are expressed as mean ± SEM. Between-group differences were analysed using one-way ANOVA followed by post-hoc Tukey's multiple comparisons test, except for non-parametric variables, (†) in which Kruskal-Wallis test followed by Dunn's multiple comparisons test was performed. Differences compared to control group are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. QST: quantitative sensory test; MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively; s: seconds, JND: Just Noticeable Differences.

6.5.1 Quantitative Sensory Testing

Higher thresholds were found in patient groups compared to the control group for QST testing in all modalities except for NMHE patients in Heat Pain 5.0 threshold when stimulus was applied on the foot and Cooling Detection time on the hand (Table 8). Significant differences were found between patient groups in Cooling Detection ($p = 0.04$), Heat Pain 0.5 ($p = 0.01$) and Heat Pain 5.0 ($p = 0.04$) when the foot was the test site. The proportion of patients in which over half the tests were outside the normal range was significantly higher ($p = 0.007$) in MHE (67%) than in NMHE patients (26%). Also, patients took longer to finish tests in general; control subjects finished each test in around 120s, the NMHE group took slightly longer (125–180 s) and the MHE group longer still (130–220 s). There were significant differences between NMHE and MHE patients in Vibration Detection time ($p = 0.04$), Cooling Detection time ($p = 0.01$) and Heat Pain time ($p = 0.01$) when the foot was the test site (Table 8).

6.5.2 Autonomic Function Testing

The amplitude of SSR was significantly reduced in patients with MHE compared to controls ($p < 0.01$) and significant differences were found between MHE and NMHE patients ($p < 0.05$). Regarding autonomic function, there was a general decrease in all parameters of heart rate variability (R-R interval) in patients, being significantly reduced in NMHE patients compared to controls in the hyperventilation test ($p < 0.05$) (Table 8). The prevalence of liver cirrhosis in our population was greater in men than in women, so the number of women in patient groups was low. Given the difficulty of recruiting healthy controls for our study, in order to have a number of subjects that could allow an

appropriate statistical analysis, we decided to include the subjects shown Table 5. Although there was a higher proportion of women in the control group than in the patient groups (Table 5), no significant differences were found between males and females in the variables analysed (Table 9).

Table 9. QST and Autonomic Testing parameters comparing males and females in the control group

QST parameters	Test site	Males	Females	P-values
Vibration Detection (JND)	hand	6.0±0.9	7.0±0.5	0.332
	foot	13.0±1.0	13.2±0.9	0.887
Cooling Detection (JND)	hand	6.7±0.5	6.8±0.4	0.864
	foot	7.9±0.4	8.3±0.6	0.660
Heat Pain 0.5 (JND)	hand	15.5±1.0	16.6±0.8	0.377
	foot	17.2±0.8	18.2±0.5	0.252
Heat Pain 5.0 (JND)	hand	20.2±0.9	19.9±0.7	0.792
	foot	21.5±0.6	21.2±0.5	0.748
Vibration Detection time (s)	hand	128.56±2.9	127.16±1.5	0.637
	foot	126.5±2.6	128.0±2.6	0.712
Cooling Detection time (s)	hand	143.9±2.0	138.8±1.6	0.060
	foot	144.0±3.1	143.6±3.3	0.942
Heat Pain time (s)	hand	113.9±18.2	110.0±10.4	0.843
	foot	130.5±15.3	121.5±12.2	0.658
Autonomic testing				
R-R Interval variation (%)	Basal	4.7±2.7	2.8±0.6	0.559
	Hyperventilation	18.2±4.9	9.3±1.7	0.163
	Valsalva	14.6±7.9	11.9±1.7	0.759
	Orthostatic test	14.5±8.1	6.2±2.2	0.176
Cutaneous sympathetic response	Amplitude	5.1±1.7	3.8±0.5	0.351
	Latency	1.33±0.02	1.42±0.05	0.362

Values are expressed as mean ± SD. Differences between groups were analysed by Student's T-test. Abbreviations: QST, quantitative sensory test; s, seconds, JND, Just Noticeable Differences.

6.6. Nerve conduction study of patients with normal sural amplitude

In order to detect early markers of MHE based on neurophysiological parameters, sural nerve amplitude was selected as a representative value of large sensory fibre impairment. Table 10 shows the results of nerve conduction studies in controls and patients with normal amplitude of sural nerve. The sural nerve amplitude was chosen as it is the most distal nerve studied in lower limbs and one of the first nerves to be affected in polyneuropathies. Sural nerve amplitude values were considered normal or

pathological according to reference values from the database of our laboratory (cut-off: 15µV). Sural nerve amplitude was affected in 42% of NMHE patients and 40% of patients with MHE, indicating an impairment of the large sensory fibre.

Table 10. Results of sensory and motor nerve conduction with normal sural nerve

Parameters	Control	NMHE patients P vs. Control	MHE patients P vs. Control	MHE patients P vs. NMHE	ANOVA Global P-values
Sensory nerve conduction					
Ulnar sensory nerve amplitude (µV)	13.1±1.4	9.8±0.6	10.0±1.0	ns	0.042
Radial sensory nerve amplitude (µV)	31.5±2.0	24.0±1.8*	33.2±2.2	0.010	0.004
Sural sensory nerve amplitude (µV)	26.5±2.1	24.5±1.7	20.6±0.9*	ns	0.059
Superior peroneal amplitude (µV)	18.2±1.3	13.3±1.3	15.5±0.7*	ns	0.020
Ulnar sensory nerve latency (ms)	1.68±0.03	1.88±0.04**	1.93±0.05**	ns	0.001
Radial sensory nerve latency (ms)	1.28±0.03	1.43±0.05	1.50±0.05*	ns	0.009
Sural sensory nerve latency (ms)	1.74±0.05	1.81±0.06	1.94±0.05	ns	0.092
Superior peroneal latency (ms)	1.92±0.06	2.05±0.08	2.14±0.08	ns	0.202
Ulnar sensory nerve conduction velocity (m/s)	58.1±0.8	56.3±0.8	54.6±0.7*	ns	0.029
Radial sensory nerve conduction velocity (m/s)	66.0±1.1	62.4±1.0	61.9±1.3	ns	0.028
Sural sensory nerve conduction velocity (m/s)	58.9±1.1	58.2±0.9	56.5±1.1	ns	0.342
Superior peroneal conduction velocity (m/s)	56.5±0.8	56.4±0.9	55.9±1.0	ns	0.921
Motor nerve conduction					
Ulnar motor nerve amplitude (µV)	14.6±0.7	14.3±0.5	14.9±0.7	ns	0.799
Peroneal motor nerve amplitude (µV)	7.4±0.5	7.9±0.4	7.0±0.5	ns	0.475
Tibial motor nerve amplitude (µV)	17.4±1.1	15.6±1.0	18.8±1.9	ns	0.239
Ulnar motor nerve latency (ms)	2.18±0.06	2.52±0.07**	2.69±0.17*	ns	0.002
Peroneal motor nerve latency (ms)	3.29±0.11	3.56±0.09	3.52±0.16	ns	0.185
Tibial motor nerve latency (ms)	3.39±0.12	3.43±0.10	3.42±0.10	ns	0.953
Ulnar motor nerve conduction velocity (m/s)	61.3±1.0	56.2±1.0**	53.1±0.4**** 43.0±0.8**	0.018	<0.001
Peroneal motor nerve conduction velocity (m/s)	47.4±1.1	43.9±0.7*		ns	0.004

Values are expressed as mean ± SEM. MHE, NMHE, patients with and without minimal hepatic encephalopathy, respectively. Differences between groups were analysed using one-way ANOVA followed by post-hoc Tukey's multiple comparisons test. Differences compared to control group are indicated by asterisks: * p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Patients with MHE presented longer latencies than controls in ulnar and radial sensory nerves. Ulnar motor nerve latency was increased in both NMHE and MHE patients compared to controls, whereas the conduction velocities of ulnar and peroneal

motor nerves were decreased in both groups of patients compared to controls, being significantly lower in MHE than in NMHE patients in ulnar motor nerve.

6.6.1 Study of small calibre fibres in patients with normal sural amplitude

Table 11 shows results of QST and autonomic function in patients with normal sural amplitude. These results are described in detail further on.

Table 11. Study of small calibre fibres in patients with normal sural amplitude

QST Parameters		Controls	NMHE Patients p vs Control	MHE Patients p vs Control	MHE Patients p vs. NMHE	ANOVA Global p Values
Vibration	hand	7 ± 0.5	9 ± 0.5 **	9.3 ± 0.7 *	ns	0.0008
Detection	foot	13 ± 0.6	16 ± 0.5 *	17 ± 0.8 **	ns	0.002
(JND)						
Cooling	hand	7 ± 0.3	9.4 ± 0.6 ***	11 ± 1 ****	ns	<0.001
Detection	foot	8 ± 0.4	11 ± 1 ***	15 ± 1.4 ****	0.004	<0.001
(JND)						
Heat Pain	hand	16 ± 0.5	17.5 ± 0.8	19 ± 1 *	ns	0.04
0.5 (JND)	foot	18 ± 0.4	19 ± 0.5	20 ± 0.5 **	0.06	0.01
Heat Pain	hand	20 ± 0.5	22 ± 0.6 *	22 ± 0.4	ns	0.013
5.0 (JND)	foot	21 ± 0.3	21 ± 0.2	23 ± 0.3	0.04	0.04
Vibration	hand	127 ± 1	132 ± 3	135 ± 3 *	ns	0.004
Detection	foot†	127 ± 2	134 ± 3 *	137 ± 2 **	ns	0.001
time (s)						
Cooling	hand†	141 ± 1	149 ± 7	156 ± 5 *	ns	0.014
Detection	foot†	144 ± 2	156 ± 8	205 ± 22 ****	0.007	<0.0001
time (s)						
Heat Pain	hand	115 ± 8	166 ± 16 **	167 ± 20 *	ns	0.014
time (s)	foot	125 ± 8	153 ± 8	160 ± 16	ns	0.06
Autonomic testing						
R-R	Basal†	3.7 ± 0.7	4.3 ± 0.9	1.8 ± 0.1 *	0.007	0.008
Interval	Hyperventilation	9.5 ± 2.0	6.7 ± 1.3	6.8 ± 2	ns	0.15
variation	Valsalva	12 ± 2	11 ± 2	10 ± 3	ns	0.95
(%)	Orthostatic test	5 ± 1	6.9 ± 1.4	2.6 ± 0.4	0.04	0.05
Sympathetic	Amplitude	4.6 ± 0.5	3.1 ± 0.4 *	1.4 ± 0.2 ****	0.03	0.0001
skin	Latency	1.4 ± 0.02	1.5 ± 0.3*	1.5 ± 0.06 *	ns	0.04
response						

Values are expressed as mean ± SEM. Between-group differences were analysed using one-way ANOVA followed by post-hoc Tukey's multiple comparisons test, except for non-parametric variables, (†) in which Kruskal-Wallis test followed by Dunn's multiple comparisons test was performed. Differences compared to control group are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. QST: quantitative sensory test; MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively; s: seconds, JND: Just Noticeable Differences.

6.6.2 QST results in patients with normal sural amplitude

Results in vibration and cooling tests demonstrated higher levels of JND for both MHE and NMHE groups when compared to controls, being higher still when the foot was the test site (Figure 18A, B). Significant differences were found between MHE and NMHE when the test site was the foot in the modalities of cooling (p=0.004). Regarding Heat Pain detection, MHE patients showed significantly higher JND than controls for Heat Pain test 0.5 in both hand (p<0.05) and foot (p<0.01), with a trend to be higher than NMHE patients when the foot was the test site (p=0.06). There was also a higher sensitivity in foot in MHE patients compared to NMHE for Heat Pain 5.0 test (p=0.04) (Table 11; Figure 18C).

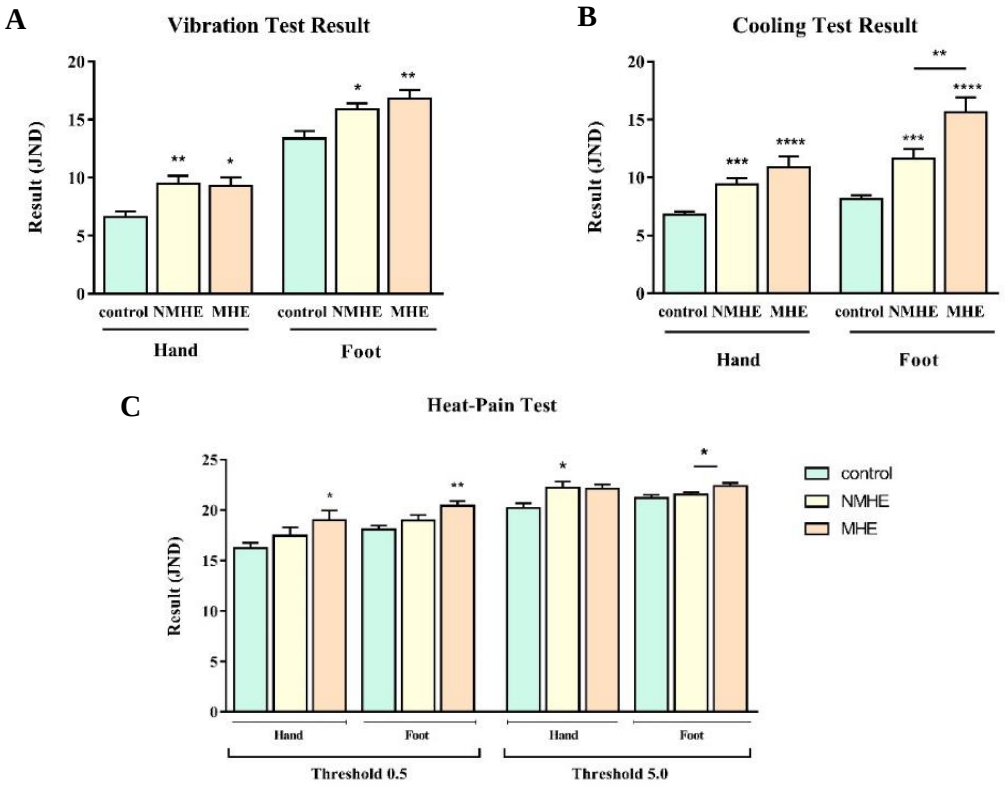

Figure 18. QST Results in cirrhotic patients with normal sural nerve amplitude. A. Vibration Test Result. B. Cooling Test Result. C. Heat-Pain Test. JND: Just Noticeable Differences. MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively. Results are mean ± SEM. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.

Comparison of alcoholic and other etiology in QST results in patients with normal sural amplitude nerve was analysed (Table 12) as was the contribution of liver disease (Table 13) and diabetes (Table 14). It was found that none of these factors influenced QST results.

Table 12. Comparison of QST parameters between patients with alcoholic etiology and with other etiologies, in the group of patients with normal sural nerve.

		Etiology		
QST parameters	**Test site**	**alcohol**	**other**	**P-values**
Vibration Detection (JND)	hand	9 ± 4	9.7 ± 2	0.62
	foot	16.2 ± 2.3	16.5 ± 2.4	0.72
Cooling Detection (JND)	hand	9.5 ± 3	10.5 ± 3	0.35
	foot	12.4 ± 5	13.2 ± 4	0.61
Heat Pain 0.5 (JND)	hand	19.4 ± 3.5	17.4 ± 3	0.10
	foot	19.8 ± 1.5	19.7 ± 2	0.92
Heat Pain 5.0 (JND)	hand	22 ± 2.4	22 ± 2.2	0.55
	foot	22 ± 1.2	22 ± 1	0.81
Vibration Detection time (s)	hand	132 ± 10	134 ± 12	0.67
	foot	133 ± 7	136 ± 11	0.44
Cooling Detection time (s)	hand	157 ± 34	148 ± 14	0.34
	foot	183 ± 80	170 ± 30	0.56
Heat Pain time (s)	hand	163 ± 55	167 ± 77	0.87
	foot	165 ± 63	152 ± 46	0.55

Values are expressed as mean ± SD. Differences between groups were analysed by Student's T-test. QST, quantitative sensory test; s, seconds, JND, Just Noticeable Differences.

Table 13. Contribution of liver disease severity to QST results observed in patients with normal sural nerve classified according to Child-Pugh score.

		Child-Pugh		
QST parameters	**Test site**	**A**	**B**	**P-values**
Vibration Detection (JND)	hand	9.5 ± 2.8	8.9 ± 2.3	0.67
	foot	16.2 ± 2.3	17.1 ± 2.1	0.46
Cooling Detection (JND)	hand	9.9 ± 3	10.5 ± 4	0.67
	foot	13.3 ± 4	10.8 ± 2	0.21
Heat Pain 0.5 (JND)	hand	18 ± 3.5	19 ± 1.5	0.27
	foot	19.7 ± 2	19.8 ± 0.5	0.93
Heat Pain 5.0 (JND)	hand	22.4 ± 2.4	21.6 ± 1	0.49
	foot	22 ± 1	22 ± 1.2	0.96
Vibration Detection time (s)	hand	134 ± 11	130 ± 10	0.50
	foot	135 ± 10	133 ± 7	0.73
Cooling Detection time (s)	hand	153 ± 26	151 ± 13	0.87
	foot	179 ± 61	157 ± 14	0.42
Heat Pain time (s)	hand	168 ± 72	154 ± 45	0.69
	foot	154 ± 50	176 ± 77	0.46

Values are expressed as mean ± SD. Differences between groups were analysed by Student's T-test. Abbreviations: QST, quantitative sensory test; s, seconds, JND, Just Noticeable Differences.

Table 14. Comparison of QST parameters and autonomic testing in patients with normal sural nerve amplitude with and without diabetes.

QST parameters	Test site	Without Diabetes	With Diabetes	P-values
Vibration Detection (JND)	hand	9.6 ± 3	9 ± 3	0.63
	foot	16 ± 2	15 ± 3	0.25
Cooling Detection (JND)	hand	10 ± 3	10 ± 3	0.98
	foot	12.5 ± 4	13.7 ± 4	0.51
Heat Pain 0.5 (JND)	hand	17.5 ± 3.4	20 ± 3	0.09
	foot	19.7 ± 2	19.2 ± 1	0.64
Heat Pain 5.0 (JND)	hand	22 ± 2	23 ± 3	0.15
	foot	21.8 ± 1	22 ± 1	0.63
Vibration Detection time (s)	hand	134 ± 11	131 ± 12	0.55
	foot	135 ± 10	133 ± 10	0.68
Cooling Detection time (s)	hand	151 ± 26	153 ± 17	0.89
	foot	176 ± 59	171 ± 41	0.83
Heat Pain time (s)	hand	162 ± 63	180 ± 78	0.55
	foot	163 ± 56	140 ± 38	0.33

Values are expressed as mean ± SD. Differences between groups were analysed by Student's T-test. Abbreviations: QST, quantitative sensory test; s, seconds, JND, Just Noticeable Differences.

6.6.3 Autonomic Testing in patients with normal sural amplitude

SSR showed significant differences in patients with and without MHE when compared to controls in both latency and amplitude. Also, differences between patient groups, MHE and NMHE, were found in amplitude values (p=0.03) being significantly lower for MHE patients (Figure 19; Table 11).

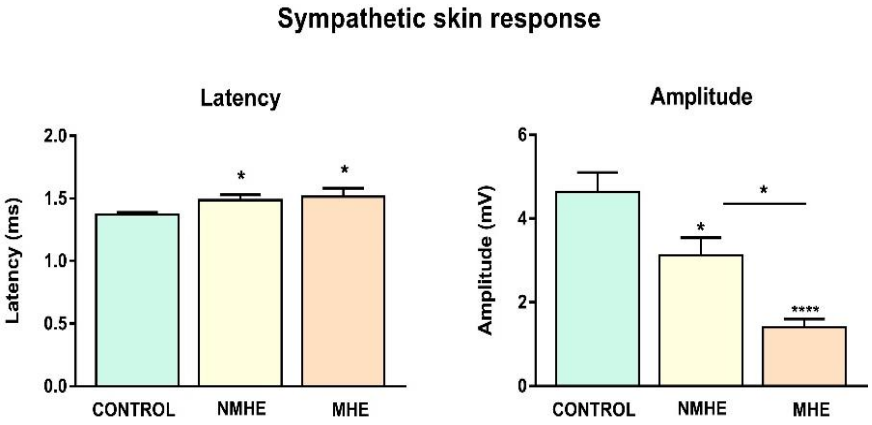

Figure 19. Sympathetic skin response. Cirrhotic patients with and without MHE show alterations in sympathetic skin response. Significant higher latencies are found for both patient groups compared to controls. Significant lower amplitude values are found in both patient groups compared to controls being significantly different between MHE and NMHE patients. MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively. Results are mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

HRV also showed differences between patients with and without MHE in basal and orthostatic test results (Figure 20). No significant differences were found between controls and patients in any of the HRV tests except for basal where MHE patients R-R interval variation was significantly lower.

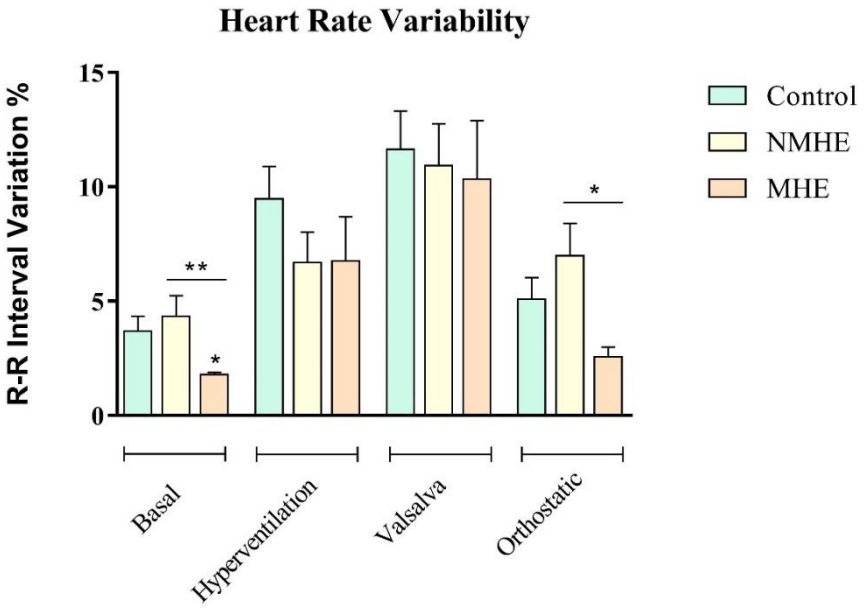

Figure 20. Heart Rate Variability. MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively. Results are mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Autonomic testing was compared between patients by etiology (Table 15), liver disease severity (Table 16) and presence of diabetes (Table 17). No significant differences were found between: patients with alcoholic etiology and with other etiologies, patients with liver severity score, Child-Pugh A and B, nor between patients with and without diabetes.

Table 15. Comparison autonomic testing between patients with alcoholic etiology and with other etiologies, in the group of patients with normal sural nerve.

Autonomic testing		Etiology		
		alcohol	other	P-values
R-R Interval variation (%)	Basal	3.6 ± 3.8	3.3 ± 2.7	0.80
	Hyperventilation	5.1 ± 3.7	8.2 ± 7	0.17
	Valsalva	11.4 ± 9.3	10.6 ± 7	0.79
	Orthostatic test	4.9 ± 5.2	5 ± 4.9	0.96
Sympathetic skin response	Amplitude	1.9 ± 1.5	2.8 ± 1.6	0.13
	Latency	1.5 ± 0.2	1.5 ± 0.2	0.48

Values are expressed as mean ± SD. Differences between groups were analysed by Student's T-test.

Table 16. Contribution of liver disease severity to autonomic testing results observed in patients with normal sural nerve classified according to Child-Pugh Score.

Autonomic testing		Child-Pugh		
		A	B	P-values
R-R Interval variation (%)	Basal	3.2 ± 3	4.6 ± 5	0.47
	Hyperventilation	6 ± 4	11 ± 10	0.33
	Valsalva	11 ± 8	13 ± 9	0.56
	Orthostatic test	5 ± 5	4 ± 2	0.49
Sympathetic skin response	Amplitude	2.6 ± 1.5	1.8 ± 2	0.36
	Latency	1.4 ± 0.2	1.7 ± 0.2	0.06

Values are expressed as mean ± SD. Differences between groups were analysed by Student's T-test.

Table 17. Comparison of autonomic testing in patients with normal sural nerve amplitude with and without diabetes.

Autonomic testing		Without Diabetes	With Diabetes	P-values
R-R Interval variation (%)	Basal	3.3 ± 2.7	3.6 ± 4.5	0.85
	Hyperventilation	7 ± 6	5 ± 4	0.44
	Valsalva	10.6 ± 7	11 ± 10	0.90
	Orthostatic test	5.7 ± 5.3	2.4 ± 2	0.12
Sympathetic skin response	Amplitude	2.9 ± 2	1.6 ± 1	0.10
	Latency	1.5 ± 0.2	1.5 ± 0.2	0.91

Values are expressed as mean ± SD. Differences between groups were analysed by Student's T-test.

6.7 Correlation of QST and autonomic system parameters with performance in psychometric tests

QST parameters correlate with psychometric testing (Table 18). There was significant correlation between PHES, the golden standard in detecting MHE, and Cooling Detection on the hand, Heat Pain 0.5 on the foot and Vibration Detection and Cooling Detection times, on both hand and foot. Cooling Detection on the foot, the QST test which found the strongest differences between patient groups, correlates with Stroop test, D2 test, Oral SDMT, Digital Span and Letter-Number Sequencing. Cooling Detection on the hand also correlates significantly with these tests (Table 18).

The Stroop test, which measures selective attention, showed a negative correlation with the Cooling Detection Thresholds in the hand and foot, and also in the detection of vibration in the foot. Moreover, the Stroop test also showed negative correlations with the detection times in the three QST tests.

The d2 test assesses selective/sustained attention and mental concentration. Higher detection thresholds in vibration and cooling correlate with lower effectiveness and concentration in the d2 test, as indicated by the negative correlations found. In addition, patients with a lower concentration in the d2 test required longer detection times in the three QST tests.

There were significant correlations between sympathetic skin response amplitude and PHES score ($r = 0.43$; $p = 0.02$), and Stroop, congruent and neutral tasks ($r = 0.441$; $p = 0.027$, and $r = 0.51$; $p = 0.01$, respectively).

Lower mental processing speed, as measured by the oral SDMT test, and lower working memory, as measured by the digit span and letter-number sequencing tests, correlate with higher Cooling Detection Thresholds, as well as longer Cooling Detection times especially in the foot (Table 18).

The correlation between motor coordination tests and QST parameters was also analysed. Only weak correlations were found between the coordination and Heat Pain 0.5 on foot, and the Cooling Detection time in the hand.

6.8 Correlations of QST parameters with biomechanical parameters

Correlations of QST parameters with biomechanical parameters are shown in Table 19. VDT hand time correlates with lateral pincer strength right and left. VDT on the hand and HPDT time for hand correlates with global gait results. CDT results on the foot correlate with strength grip results of both hands and HPDT 5.0 on the foot also correlates with grip strength on the right hand.

6.9. Correlations of QST parameters with biochemical parameters

Significant correlations of QST parameters and blood levels of IL-6 and ammonia are shown in Table 20. Higher IL-6 levels correlate with higher Cooling Detection Thresholds on foot and in heat-pain detection 0.5 on hand, as indicated by the significant positive correlations found.

Table 18. Correlations of QST parameters with psychometric tests.

QST Parameters		PHES Score	Stroop Test	d2 Test		Oral SDMT	Digit Span	Letter-Number Sequencing	Coordination Tests
				d2-TOT	d2-CON				
Vibration Detection (JND)	hand						-0.23 (0.06)	-0.40 (0.002)	
	foot		-0.31 (0.01)	-0.27 (0.03)	-0.26 (0.04)	-0.23 (0.06)	-0.39 (0.001)	-0.29 (0.02)	0.23 (0.07) §
Cooling Detection (JND)	hand	-0.33 (0.004)	-0.47 (<0.001)		-0.31 (0.01)	-0.36 (0.002)	-0.25 (0.04)	-0.38 (0.002)	0.23 (0.05)
	foot		−0.37 (0.002	−0.25 (0.04)	−0.28 (0.02)	−0.36 (0.002)	−0.29 (0.02)	−0.30 (0.02)	
Heat Pain 0.5 (JND)	hand		−0.21 (0.08)	−0.25 (0.04) ‡		−0.27 (0.03)			
	foot	−0.34 (0.005)	−0.22 (0.07)						0.29 (0.02) §
Heat Pain 5.0 (JND)	hand					−0.24 (0.06)	−0.32 (0.01)	−0.28 (0.03)	
	foot								
Vibration Detection time (s)	hand	−0.32 (0.008)	−0.30 (0.02)	−0.34 (0.006)	−0.34 (0.009)			−0.25 (0.06)	0.24 (0.06) §
	foot	−0.35 (0.003)	−0.24 (0.05)			−0.25 (0.06)			0.24 (0.06) §
Cooling Detection time (s)	hand	−0.22 (0.06)	−0.26 (0.03)	−0.26 (0.04) ‡		−0.38 (0.001)			0.25 (0.04)
	foot	−0.24 (0.04)	−0.29 (0.01)	−0.31 (0.01)	−0.32 (0.009)	−0.34 (0.004)	−0.41 (0.001)	−0.40 (0.001)	
Heat Pain time (s)	hand		−0.25 (0.04) †		−0.24 (0.05)	−0.33 (0.006)	−0.34 (0.006)	−0.30 (0.02)	
	foot			−0.29 (0.02)	−0.29 (0.02)				

The r values (p-values) of the Pearson correlation analysis are shown. Significant and near-significant correlations are shown. Values for Stroop test are for Incongruent task, except for (†), which are for Neutral task. ‡ Values for d2-TR, Total number of characters processed; § values for visual-motor coordination test; values for bimanual coordination test. QST: quantitative sensory test; JND: just noticeable differences; s: seconds; PHES: Psychometric Hepatic Encephalopathy Score; d2 test: TOT: Total correctly processed; CON: Concentration performance; Oral SDMT: Symbol digit modalities test (oral version).

Table 19. Correlations of Biomechanics and QST parameters

QST Parameters		Grip mean right	Grip mean left	Lateral pinch strength right	Lateral pinch strength left	Global gait
VDT (JND)	**hand**	0.06 (0.66)	0.09 (0.51)	-0.25 (0.09)	-0.14 (0.37)	**0.35* (0.017)**
	foot	0.16 (0.25)	0.14 (0.34)	-0.2 (0.18)	-0.11 (0.49)	0.077 (0.61)
CDT (JND)	**hand**	0.19 (0.17)	0.14 (0.33)	0.05 (0.73)	0.011 (0.94)	-0.02 (0.89)
	foot	**0.31* (0.03)**	**0.35* (0.03)**	-0.01 (0.92)	-0.1 (0.54)	-0.11 (0.49)
HPDT 0.5 (JND)	**hand**	**0.3* (0.03)**	0.26 (0.07)	0.17 (0.26)	-0.16 (0.31)	0.003 (0.98)
	foot	0.28 (0.06)	0.19 (0.21)	-0.23 (0.15)	-0.19 (0.26)	-0.2 (0.9)
HPDT 5.0 (JND)	**hand**	0.17 (0.24)	0.06 (0.69)	-0.15 (0.22)	-0.07 (0.69)	-0.29 (0.05)
	foot	**0.316* (0.035)**	0.14 (0.36)	-0.2 (0.19)	0.06 (0.7)	0.13 (0.42)
VDT time (s)	**hand**	0.15 (0.3)	0.17 (0.25)	**-0.37* (0.01)**	**-0.39* (0.01)**	-0.13 (0.39)
	foot	0.83 (0.56)	-0.43 (0.77)	-0.003 (0.99)	-0.05 (0.75)	-0.16 (0.28)
CDT time (s)	**hand**	0.26 (0.07)	0.26 (0.06)	0.13 (0.4)	-0.11 (0.49)	0.15 (0.32)
	foot	0.09 (0.52)	0.13 (0.36)	-0.04 (0.81)	-0.29 (0.06)	-0.02 (0.92)
HPDT time (s)	**hand**	0.19 (0.16)	0.99 (0.48)	0.12 (0.4)	-0.05 (0.76)	**-0.35* 0.015**
	foot	0.23 (0.11)	0.09 (0.52)	-0.2 (0.18)	-0.16 (0.31)	0.03 (0.86)

The r values (p-values) of the Pearson correlation analysis are shown. JND: Just Noticeable Differences; s: seconds; VDT: Vibration Detection Threshold; CDT: Cooling Detection Threshold; HPDT: Heat Pain Detection Threshold. *P-value<0.05; **P-value<0.01; ***P-value <0.001; ***P-value<0.0001.

Longer detection times in all three QST tests correlate with higher levels of both IL-6 and ammonia. The duration of CDT and VDT tests, when on the foot was the test site, significantly correlate with both IL6 and ammonia levels. Duration of HPDT testing on the hand also correlates with both biochemical parameters, whilst on the foot correlates only with ammonia levels.

Table 20. Correlations of QST parameters with biochemical parameters.

QST Parameters	Test Site	IL6 (pg/ml)	Ammonia (µM)
CDT (JND)	**foot**	0.347 (0.004)	
HPDT 0.5 (JND)	**hand**	0.273 (0.02)	
VDT time (s)	**foot**	0.257 (0.03)	0.350 (0.004)
CDT time (s)	**foot**	0.417 (<0.001)	0.312 (0.008)
HPDT time (s)	**hand**	0.318 (0.007)	0.397 (0.001)
	foot		0.376 (0.002)

The r values (p-values) of the Pearson correlation analysis are shown. Significant and near-significant correlations are shown. JND: Just Noticeable Differences; s: seconds; VDT: Vibration Detection Threshold; CDT: Cooling Detection Threshold; HPDT: Heat Pain Detection Threshold.

6.10 Correlations of QST parameters with SSR

Sympathetic skin response also correlates with QST. Amplitude correlates with vibration threshold in foot ($r = 0.361$; $p = 0.018$) and cooling threshold in hand ($r = -0.380$; $p = 0.013$), and also with Heat Pain Detection 0.5 in foot ($r = -0.317$; $p = 0.049$).

6.11 Predictive Capacity of QST parameters for detecting MHE

A receiver operating characteristic curve (ROC) analysis was conducted (Figure 21). Results show that Cooling Detection and Cooling Detection time, on the foot as the test site, has a significant predictive capacity for detecting MHE (Table 20), with the following area under the ROC curve (AUC):

- Cooling Detection on foot (JND): AUC: 0.759; 95% CI (0.565–0.952). Sensitivity was 73% and specificity 70% at the cut-off value of 12.95 JND.
- Cooling Detection time for foot (s): AUC: 0.838; 95% CI (0.695–0.980). Sensitivity was 75% and specificity 68.4% at the cut-off value of 158.5 seconds.

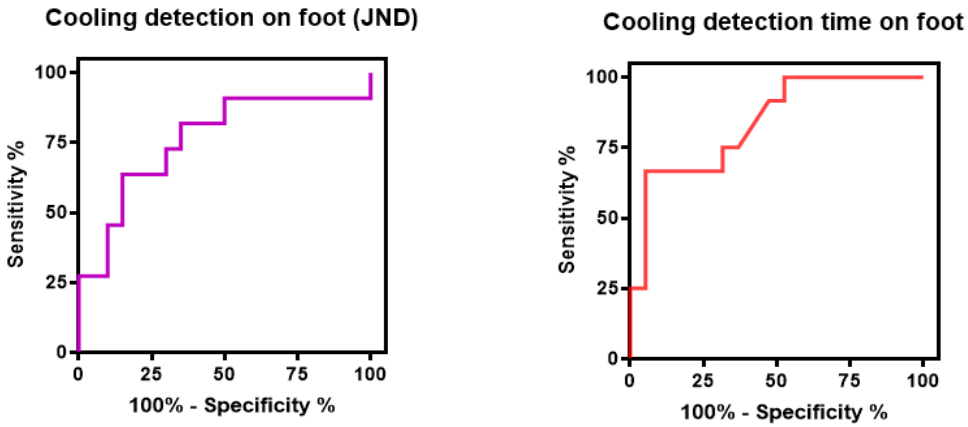

Figure 21. Cooling Detection on the foot has a significant predictive capacity for detecting MHE. Roc analyses show the following area under the ROC curve for Cooling Detection (AUC): 0.759; 95% CI (0.565–0.952) and AUC: 0.838; 95% CI (0.695–0.980) for Cooling Detection time. JND: Just Noticeable Differences.

Table 21. ROC curve parameters determining the predictive capacity of QST tests.

Parameters	AUROC (95% CI)	p value	Cutt off point	Sensitivity (%)	Specificity (%)
Cooling Detection foot	0.759 (0.565–0.952)	0.018	12.95 JND	72.73	70
Cooling Detection time	0.837 (0.695–0.980)	0.0018	158.5 s	42.81	68.42

Receiver operating characteristic curve (ROC) for sensitivity and specificity of QST cooling parameters for the diagnosis of MHE in cirrhotic patients. AUROC: Area under the curve; CI: Interval of confidence.

VII Discussion

VII. Discussion

The main results found in this study are:

- Hyposensitivity is present in cirrhotic patients but is higher in patients with MHE.
- MHE patients have impaired thermal sensitivity, both in cooling and Heat Pain detection.
- This impairment correlates with worse performance in attention, mental processing speed, and working memory tests and also with higher blood levels of ammonia and IL6.
- Thermal sensitivity and autonomic function, involving small calibre nerve fibres, are early alterations associated with MHE, which appear before sural nerve amplitude (large nerve fibre) is altered.

The present results provide first time evidence of impaired detection of cold and temperature changes in MHE patients compared to those without MHE. Although lower sensitivity to vibration and thermal stimuli was also observed in the two patient groups compared to the control group in both hand and in foot, MHE patients had a greater hyposensitivity when the foot was the test site. The different results found in lower limbs and upper limbs can be explained due to the characterization of toxic-metabolic polyneuropathies which can be found as a comorbidity of liver diseases and are length-dependent, this means lower distal limbs are the first to be affected. Alterations reported in hepatic encephalopathy are classified within the chronic axonal polyneuropathies of toxic-metabolic origin, which these are characterized by a length-dependent, die-back degeneration of axons, initially affecting the sensory fibres and with a pattern of distal

predominance. In nerve conduction studies, the main finding is a decrease of the SNAP (sensory nerve action potential) and CMAP (compound muscle motor action potential) amplitudes, affecting the lower extremities more than upper extremities, with normal conduction velocity and distal latency. Although conduction velocity can be reduced up to 70–80% of reference values if there is a decrease in CMAP amplitude greater than 50%, which is associated to a selective loss of fast-conducting fibres (Katirji, 2007).

The results found in this study contrast with the previous study from Brenner et al. (2015) where the hand was used as the test site and therefore results found impaired thermal perception in grade 2 HE patients, but no differences in MHE patients. They concluded that these alterations emerge mainly in advanced stages of the disease. Like Brenner et al., the present study also found no differences between the two patient groups when the test was performed on the hand. However, by testing the foot a greater hyposensitivity in MHE patients was found, suggesting that in these patients, the distal sensory fibres become impaired earlier than the proximal ones found in the hands, which do not deteriorate until more advanced grades of HE (Brenner et al., 2015). The differences found between hand and foot test sites indicate that the foot is more sensitive in distinguishing between cirrhotic patients with or without MHE, while the hand seems to have more sensitivity in distinguishing cirrhotic patients who have not yet developed MHE from control subjects.

Peripheral neuropathy is a frequent complication of liver cirrhosis (Chaudhry et al., 1999b; Jain et al., 2014; Kharbanda et al., 2003), that can affect more than 70% of cirrhotic patients. In in this study, however, only 38% of cirrhotic patients presented peripheral neuropathy. This lower incidence could be due to differing cirrhotic patient

profiles, given that previous studies found this high incidence in patients with end-stage liver disease awaiting liver transplantation, whereas patients in the present study all had compensated cirrhosis. Moreover, neuropathy grade has been linked to liver disease severity, with a higher neuropathy score in Child-Pugh class C patients than those in A and B (Chaudhry et al., 1999). In this study, nonetheless, no patients were in Child Pugh C grade, in fact most were in Child-Pugh A (74%), so we can reasonably rule out liver disease severity as underpinning the effects observed in this study. Patients with liver disease are at high risk of developing other metabolic syndromes such as diabetes, in which small fibre neuropathy is often concomitant. In this study we show that most alterations observed in small fibres were not influenced by diabetes.

The studies of the autonomic nervous system (RR interval, sympathetic skin response) and of thermal sensitivity are neurophysiological studies useful for evaluating function of small fibres. The term small fibre neuropathy refers to a group of neuropathies characterized by a selective or predominant disorder of the poorly myelinated peripheral A delta afferent fibres and the unmyelinated C fibres (Vollert et al., 2017). In the somatosensory nervous system, these fibres transmit information of temperature, pain, and itching, and in the neurovegetative nervous system they are involved in sudomotor, thermoregulatory, cardiovascular, gastrointestinal, urogenital, and other functions (Mathias & Bannister, 2013).

In this study we show that MHE is more associated with alterations in small fibre function (cooling and heat pain thresholds) rather than large fibre function (vibration threshold). Patients with MHE with normal sural conduction, do not present alterations in large nerve fibres; however, in our study, we found that they present alterations in

thermal sensitivity and autonomic function, implying alterations in small nerve fibres which are involved in these functions. This would indicate that in MHE patients, alterations in thermal perception and autonomic nervous system precede alterations in large fibres, given the impairment in QST parameters and RR interval variation found in MHE patients compared to NMHE patients.

The autonomic or vegetative nervous system is involved in regulating involuntary functions of the organism, maintaining internal homeostasis, and adapting responses to variations in the external and internal environment. The sudomotor function is part of the thermoregulation system, a complex homeostatic system integrated in the hypothalamus. By studying this system, we can assess possible abnormalities in autonomic nervous system function. Within the patient group with normal sural nerve amplitude (no distal large fibre impairment) sympathetic skin response amplitude was altered in 36% of MHE patients compared to 11% in NMHE patients. MHE patients also presented more changes in heart rate variability (RR interval) (small fibre involvement) in both the baseline study, at rest, and the orthostatic test or passive tilt test. This data indicates involvement of both the sympathetic and parasympathetic nervous systems in MHE. Taken together, these results further suggest that the small fibres of the autonomic nervous system (sympathetic skin response, RR interval, and thermal sensitivity) are altered in early stages of MHE while changes to the large sensory fibres (sural nerve) take place later on.

The correlations found indicate that deficits in attention, mental processing speed, and working memory are associated with an impaired QST response. Although the PHES was defined as the gold standard to detect MHE (Ferenci et al., 2002), it was later shown that it fails to detect certain mild cognitive alterations and classifies patients with these

alterations as NMHE (Felipo et al., 2012, 2014; Giménez-Garzó et al., 2017). The fact that NMHE patients also presented significant impairments in QST parameters compared to controls could be associated with the presence of an early attentional impairment undetected by PHES. Another possibility is QST maybe detecting subclinical compensatory alterations that maybe occurring as a generic response from the immune system in response to disease and peripheral inflammatory signals. Results have shown that high levels of ammonia and IL-6 can be found in both patient groups, also NMHE. In theory, the IL-6 – COX – PGE2 axis maybe playing a part in trying to prevent the upregulation of proinflammatory cytokines by impeding release of DAMPs and promoting the downregulation of inflammation. IL-1β has been shown to hyperpolarize a population of neurons in the hypothalamus reducing thermosensitivity produced by PGE2 (Tabarean, 2018). This leads to a shift in the thermostatic set point that has been shown to reduce inflammation, promote tolerance and prevent excessive tissue damage (Schieber & Ayres, 2016) and therefore it is possible that this could be an initial allostatic result to the presence of hyperammonaemia and chronic inflammation. This might explain why hyposensitivity in thermal results can also be found in cirrhotic patients without MHE. PGE2, has also been reported to play a crucial part in mediating immunosuppression in decompensated cirrhotic patients (Arroyo & Moreau, 2014; China et al., 2018; O'Brien et al., 2014) and has been found in high cerebral concentrations in animal models (Rodrigo et al., 2010). PGE2 has also been related to cognitive impairment by way of the COX-2 - PGE2 - EP2 signalling pathway which regulates long-term potentiation (LTP) under physiological conditions. However, in pathological states, LPS

impairs LTP and causes cognitive problems via excessive upregulation of COX-2 and EP2 receptor (Jiang et al., 2020).

This theory further complicates the difficulty to ascertain whether the sensory impairments found are central or peripheral: both components are likely to be altered. The correlation with the Stroop test suggests that the attention needed to synchronize activity between high- and low-order areas of the parietal cortex to enhance relevant sensory signals may be deficient. This also points to central impairment, in agreement with previous studies in which patients with overt HE showed alterations in thermal sensitivity strongly correlated with central impairment (Brenner et al., 2015). The significant correlations found between time detection in all QST modalities and attention tests performed could also be pointing not to a deficit in sensing (peripheral impairment), but to a deficit in reporting due to attention dysfunction (central impairment). Therefore, these results would indicate that the delayed response in thermal and vibration detection could be due to alterations in mental processing speed and attention. MHE patients experience psychomotor slowing (Butz et al., 2010), and longer reaction times, which could contribute to the longer times needed by MHE than NMHE patients to perform the QST tests, mainly when the foot was the test site.

The correlation of SSR amplitude with cooling and thermal thresholds might reflect a problem with awareness level regulation due to presence of MHE and the possible underlying pathologic mechanism common to both cognitive impairment and autonomic dysfunction.

Impaired autonomic function is present in patients with mild cognitive impairment (such as orthostatic blood pressure dysregulation) and several different types of dementia: Alzheimer's disease, frontotemporal dementia, dementia with Lewy bodies, and Parkinson's disease with dementia (Toru et al., 2018). Nicolini et al., (2014) suggested that the underlying physiopathological mechanism common to both autonomic and cognitive function could be the disruption of central autonomic control due to damage of the right insula or locus coeruleus damage. Arousal-induced SSR has been formerly associated to a right hemisphere lateralization and right anterior insula activation (Vetrugno et al., 2003). The insula also contains the somatotopic map of thermosensation and pain (Gasquoine, 2014; Uddin et al., 2017). In a previous study by magnetic resonance it was found that MHE patients showed a reduction in grey matter of the right insula, which correlated with PHES score, attention tests, and inflammation (García-García et al., 2017). This alteration found in the insula might also explain the relationship between thermosensory and autonomic impairments in cirrhotic patients with MHE.

Quantitative Sensory Testing of Cooling Detection on the foot (both JND and detection time) has a significant predictive capacity for detecting MHE, as indicated by the obtained ROC curves. Although we cannot discern whether the results of Cooling Detection time were due to a peripheral deficit in "sensing" or to a central deficit in "reporting" due to the attention dysfunction, we consider this parameter to be of value in clinical practice and a useful element to consider in the diagnosis of MHE, because although it does not pinpoint the origin of the pathophysiology, it does predict the presence of MHE. QST testing also gives us a more comprehensive way of evaluating

MHE, allowing an easy, low cost, and non-invasive way to check on a patient's evolution and incrementing opportunities to alter prognosis early on.

A limitation of this study was that there are no 'pure' cirrhotic patients because they usually present other comorbidities, such as diabetes mellitus, arterial hypertension, and /or kidney complications. MHE patients have central alterations, peripheral neuropathy (a frequent complication of liver cirrhosis) and a high probability of coinciding with other metabolic syndromes such as diabetes, which in turn, frequently present small fibre neuropathy. Consequently, it is difficult to differentiate whether the polyneuropathy present in cirrhosis is secondary to diabetes mellitus or cirrhosis. In this study, there were no significant differences between those with or without diabetes in early alterations of thermal sensitivity found in MHE patients, which would indicate a main role of MHE in these alterations.

Some medications, such as lactulose, could change the presence of MHE. As shown in Table 5, three NMHE patients (8% of total NMHE) and four MHE patients (20% of total MHE) were taking lactulose. It was reported in the literature that lactulose may improve MHE (Bajaj et al., 2010; Hudson & Schuchmann, 2019). This does not seem to be the case in our study, as four patients taking lactulose presented MHE.

Regarding the alterations in the autonomic function, it was also difficult to differentiate the contribution of liver cirrhosis from the use of medication for arterial hypertension, like beta-blockers. However, the differences in autonomic function observed between NMHE and MHE patients could not be due to beta-blockers, given that the proportion of MHE patients taking this medication were lower (5/20; 25%) than NMHE patients (15/38; 39%).

VIII Conclusion

VIII. Conclusions

- Patients with MHE show a general decrease in cognitive and sensory abilities.
- The peripheral nervous system seems to be affected early on in patients with MHE, this is a novel contribution, as previous literature determines peripheral nervous system alteration to appear in late stages of Hepatic Encephalopathy.
- MHE is more associated with alterations in small fibre function (cooling and Heat Pain thresholds) rather than large fibre function (vibration threshold)
- Small fibres of the autonomic nervous system and cooling sensitivity are mainly affected at early stages of MHE, predominantly distal and in the lower limbs, even before the large sensory fibres become altered.
- It was found that there was also an early alteration of the sympathetic skin response in MHE patients, with a lower prevalence in patients without MHE. This could be considered as an early marker of pathophysiology and could be useful for the early detection of patients who are susceptible of developing MHE.
- Deficits in attention, mental processing speed, and working memory in MHE patients are associated with an impaired QST response.
- Quantitative Sensory Testing can be useful to detect these issues early on, as well as the presence of MHE, a point at which prognosis can still be altered. This early detection can improve patient's outcome and quality of life, since treatment would likely be implemented beforehand and therefore treatment response would probably be better as the person is at an earlier stage of the disease.
- Quantitative sensory testing could be a practical and functional clinical tool to use as a complementary test to detect MHE.

IX References

IX. References

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X Indexes

10.1 Index of figures

10.2 Index of tables