TESIS DOCTORAL

CARACTERIZACIÓN CLÍNICA, GENÉTICA Y DE NEUROIMAGEN DE UNA SERIE DE PACIENTES CON ESCLEROSIS LATERAL AMIOTRÓFICA

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La Dra. M Teresa Sevilla Mantecón de la Universitat de València,

DECLARA:

Que el trabajo titulado *Caracterización clínica, genética y de neuroimagen de una serie de pacientes de Esclerosis Lateral Amiotrófica*, que presenta Juan F. Vázquez Costa para la obtención del título de doctor, se ha realizado bajo mi dirección.

Y para que así conste y tenga los efectos oportunos, firmo el presente documento.

M Teresa Sevilla Mantecón Valencia, 21 de enero de 2019

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ABREVIATURAS

ADN: ácido desoxirribonucleico

AMP: atrofia muscular progresiva

ARN: ácido ribonucleico

DFT: demencia fronto-temporal

DLFT: degeneración lobar fronto-temporal

ELA: esclerosis lateral amiotrófica

ELAc: fenotipo clásico de esclerosis lateral amiotrófica

ELP: esclerosis lateral primaria

EMG: electromiograma

FUS: *fused in sarcoma*

MNS: motoneurona superior

MNI: motoneurona inferior

TARDBP: *transactive response DNA binding protein*

TDP-43: *transactive response DNA binding protein 43 kDa*

RM: resonancia magnética

SOD1: *superoxide dismutase 1*

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1. INTRODUCCIÓN

A. ESCLEROSIS LATERAL AMIOTRÓFICA: UNA ENFERMEDAD HETEROGÉNEA

EPIDEMIOLOGÍA Y CONCEPTO CLÍNICO-PATOLÓGICO

La esclerosis lateral amiotrófica (ELA) es una enfermedad neurodegenerativa que afecta característicamente a la motoneurona superior (MNS, situada en el córtex motor) y a la motoneurona inferior (MNI, situada en el asta anterior medular) produciendo una parálisis progresiva que acaba llevando al fallecimiento, normalmente por insuficiencia respiratoria, en una media de 3 años (Brown and Al-Chalabi 2017; van Es et al. 2017).

En población occidental, el riesgo vital acumulado de padecer la ELA es de una de cada 400 mujeres y a uno de cada 350 varones a lo largo de su vida (Brown and Al-Chalabi 2017; van Es et al. 2017). En Europa, la incidencia de la enfermedad es bastante homogénea, si bien parece algo menor en nuestro mediterránea (Pradas et al. 2013). Debido a su elevada y rápida mortalidad, la prevalencia de la ELA no suele superar los 5 casos por cada 100.000 habitantes (Brown and Al-Chalabi 2017; van Es et al. 2017), por lo que se engloba dentro de las enfermedades raras (ORPHA: 803). Como corresponde a una enfermedad neurodegenerativa, el envejecimiento juega un papel fundamental en la ELA aunque, a diferencia de la enfermedad de Alzheimer, el pico de incidencia se alcanza antes de los 75 años (van Es et al. 2017).

La ELA comparte características patológicas con otras enfermedades neurodegenerativas, en concreto la presencia de degeneración neuronal y gliosis reactiva en presencia de depósitos de proteínas mal plegadas. En el caso de la ELA encontramos, en el 98% de los pacientes, depósitos citoplasmáticos (en neuronas y oligodendroglía) ubiquitinados de TDP-43 (Brettschneider et al. 2014), una característica que comparte con la degeneración lobar frontotemporal por depósitos de TDP-43 (DLFT-TDP), y particularmente (por su distribución y morfología) con el subtipo B de la DLFT-TDP (Burrell et al. 2016). En el 2% restante de los pacientes (indefectiblemente portadores de mutaciones en *SOD1* y *FUS*) encontraremos depósitos de estas proteínas en lugar de TDP-43 (Blokhuis et al. 2013). Otras proteínas como p62, optineurina o ubiquilina-2 se encuentran también frecuentemente tanto en pacientes esporádicos como genéticos, mientras que otra inclusiones (como las *dipeptide repeat proteins*)

aparecen exclusivamente en pacientes portadores de determinadas mutaciones (en este caso expansiones en *C9ORF72*) (Blokhuis et al. 2013). Rodeando a las neuronas en degeneración encontramos con frecuencia microglía activada con depósitos de hierro en su interior y astrogliosis (Philips and Rothstein 2014).

Independientemente de la naturaleza y característica de los depósitos, la afectación neuronal en la ELA se inicia, por lo general, de forma focal en el área motora y/o asta anterior, para luego extenderse por diversas regiones del sistema nervioso siguiendo un patrón más o menos característico con una particular predilección por los lóbulos frontales así como determinadas estructuras subcorticales como la sustancia reticular ascendente, núcleos pre-cerebelosos o la sustancia negra (Brettschneider et al. 2014). Esta estereotipada y característica distribución parece depender de las conectividad del córtex motor (Brettschneider et al. 2014). Así, la extensión de la patología neuronal en la ELA, como en otras enfermedades neurodegenerativas, parece ser consecuencia de la transmisión célula a célula (*prion-like*) de los agregados proteicos (Taylor, Brown, and Cleveland 2016).

La presentación clínica (región de inicio) y la progresión de la enfermedad (extensión de la debilidad) refleja estas características patológicas (Ravits 2014). Esto es, por lo general la enfermedad presenta un inicio clínico focal en forma de debilidad en una región corporal que posteriormente se extiende a otras regiones (Brettschneider et al. 2014). Además, durante el curso de la enfermedad, al menos la mitad de los pacientes de ELA presentarán cierto grado de deterioro cognitivo del espectro de la demencia frontotemporal (DFT) y hasta un 15% cumplirán criterios de DFT, como consecuencia de la extensión patológica de la enfermedad por los lóbulos frontales (Burrell et al. 2016; Strong et al. 2017; Crockford et al. 2018). Por esto, actualmente tiende a considerarse a la ELA y a la DFT como dos extremos del mismo espectro clínico-patológico (Robberecht and Philips 2013; Burrell et al. 2016) (Figura 1).

ELA ELA-DC ELA-DFT DFT-ELA DFT

Figura 1. La ELA y la DFT se consideran dos extremos del mismo espectro clínico-patológico con extremos en los que hay una afectación pura motora o cognitiva (aproximadamente el 50% de los casos) y estados intermedios en los que la afectación motora y cognitiva se presentan en un grado variable. Modificado de Robberecht and Philips, 2013.

ASPECTOS FISIOPATOLÓGICOS

Un concepto comúnmente aceptado es que la ELA sería consecuencia de la interacción de factores genéticos y ambientales en gran medida desconocidos (Zufiría et al. 2016). Estos factores actuarían durante la vida del sujeto como un proceso con múltiples etapas, de forma similar a lo que se ha propuesto en el cáncer, de forma que cuando se alcanzara determinado umbral, se desencadenaría la enfermedad (Al-Chalabi et al. 2014). En este modelo, el trasfondo genético determinaría el número de eventos patológicos posteriores necesarios para alcanzar el umbral de enfermedad (Chiò et al. 2018).

Aunque los mecanismos que llevan a la degeneración neuronal no son bien conocidos, los genes relacionados con la ELA, se agrupan en torno a tres mecanismos principales de acción (Brown and Al-Chalabi 2017): la homeostasis proteica, el metabolismo del ARN y la dinámica del citoesqueleto. Alteraciones en estos procesos, posiblemente conducen a una disfunción mitocondrial y estrés oxidativo que conducen a la muerte neuronal. Ésta, a su vez, propicia dos fenómenos que podrían colaborar a la degeneración neuronal: la reacción glial y la hiperexcitabilidad cortical. Aunque la reacción glial podría tener tanto efectos beneficiosos como perjudiciales, en el caso de la microglía la balanza parece inclinarse hacia un efecto deletéreo (Philips and Rothstein 2014) ya que, por ejemplo, determinadas variantes funcionales del CX3CR1 que favorecen la activación de la microglía se asocian a peor pronóstico (Lopez-Lopez et al. 2014). Por lo que respecta a la hiperexcitabilidad, parece secundaria a la muerte, en etapas tempranas, de interneuronas en el área motora cerebral (Geevasinga et al. 2016). La hiperexcitabilidad podría colaborar a la muerte neuronal transináptica mediante un mecanismo de excitotoxicidad glutamatérgica (Geevasinga et al. 2016).

Clínicamente, la hiperexcitabilidad cortical se traduce en fasciculaciones, hiperreflexia y espasticidad (Geevasinga et al. 2016).

Otro aspecto fisiopatológico a tener en cuenta en la ELA es el hipermetabolismo, esto es, el aumento de consumo energético basal que se traduce en una desproporcionada pérdida de peso desde fases iniciales de la enfermedad (Geevasinga et al. 2016). Las causas de este hipermetabolismo no están claras aunque podría estar relacionado con una disfunción hipotalámica (Dupuis et al. 2011).

HETEROGENEIDAD CLÍNICA Y DIAGNÓSTICO

Como hemos visto, el concepto clínico-patológico que define a la ELA es muy amplio y en este sentido engloba probablemente a varias enfermedades que, pese a compartir características clínico-patológicas, presentan una gran variabilidad fenotípica (Al-Chalabi et al. 2016b). Gran parte de esta variabilidad viene dada por diferencias en:

- La región de inicio de los síntomas. Ésta parece estar determinada por el azar y puede ser bulbar (25%) o espinal (75%) y dentro de espinal, de inicio en miembros inferiores, miembros superiores, musculatura axial o respiratoria (Ravits 2014).
- El grado de afectación de ambas motoneuronas. Como hemos señalado, la ELA se caracteriza por una afectación clínico-patológica de la MNS y MNI. Sin embargo, aproximadamente un 10% de los pacientes presentarán únicamente signos clínicos y/o neurofisiológicos de motoneurona superior (esclerosis lateral primaria, ELP) o inferior (atrofia muscular progresiva, AMP) (Al-Chalabi et al. 2016b), sin que se hayan aclarado las causas que determinan esta afectación diferencial.
- La presencia de síntomas o signos no motores. La extensión de los depósitos de TDP-43 más allá de las motoneuronas va a resultar en la presencia de síntomas o signos sensitivos o disautonómicos (van der Graaff et al. 2009), así como cognitivos o comportamentales (Strong et al. 2017). Éstos últimos son particularmente frecuentes en la ELA, pudiendo afectar hasta al 80% de los pacientes en estadios avanzados de la enfermedad (Crockford et al. 2018).

- La edad de presentación. Aunque el pico de incidencia se sitúa entre los 55 y los 75 años, aproximadamente el 10% de los pacientes debuta antes de los 35 años o después de los 80 (Al-Chalabi et al. 2016b).
- La velocidad de propagación de la enfermedad por las redes neuronales.

Todos estos factores influyen de una manera u otra en la presentación clínica y la evolución de la enfermedad, dificultando el diagnóstico y una estratificación pronóstica adecuada.

Actualmente, el diagnóstico de la ELA continúa siendo esencialmente clínico, basado en la presencia de síntomas y signos clínicos de MNS y MNI, junto con la presencia de signos neurofisiológicos compatibles de afectación de MNI (denervación aguda, reinervación crónica y fasciculaciones), la constatación de progresión clínica y la exclusión de otras causas. El número de regiones que muestran afectación de MNS y/o MNI aumenta el grado de certidumbre diagnóstica (Cortés-Vicente et al. 2017). Por ello, los criterios diagnósticos revisados de El Escorial (Brooks et al. 2000), distinguen diversas categorías diagnósticas (posible, probable con apoyo de laboratorio, probable, definida) según el número de regiones afectas clínica o electrofisiológicamente. Los más recientes criterios de Awaji incorporan las fasciculaciones, un sello distintivo de la enfermedad, para aumentar la sensibilidad diagnóstica (de Carvalho et al. 2008). Sin embargo, y como resultado de la frecuente ausencia de signos de MNS o MNI en la presentación clínica (Ravits 2014), estos criterios continúan mostrando una limitada sensibilidad por lo que resultan poco útiles en la práctica clínica. Últimamente se está trabajando en unos nuevos criterios que incluyan los avances recientes en el conocimiento de la enfermedad como la presencia de mutaciones o deterioro cognitivo, y que permitan el diagnóstico en los fenotipos restringidos (Agosta et al. 2015). Pese a todo, el papel de los biomarcadores para el diagnóstico de la ELA continúa sin definir. Así pues, el resultado de la heterogeneidad clínica y la ausencia de biomarcadores es un retraso diagnóstico medio de un año, es decir, un tercio de la expectativa de vida del paciente (Agosta et al. 2015).

Por otro lado, la estratificación pronóstica depende también, en gran medida, de parámetros clínicos, con un valor todavía limitado de los biomarcadores (Westeneng et al. 2018).

B. BASES GENÉTICAS DE LA ELA

La ELA se manifiesta como una enfermedad esporádica en el 90% de los casos y familiar en el 10% restante. Clásicamente, se consideraban casos familiares, y por tanto de causa genética, a aquellos que se presentaban en al menos dos miembros de la misma familia. Posteriormente se amplió el concepto también a aquellos pacientes con antecedentes familiares de demencia frontotemporal (Byrne et al. 2011) y recientemente se ha propuesto la inclusión de otras enfermedades neuropsiquiátricas (en particular la esquizofrenia) que están epidemiológica y genéticamente relacionadas con la ELA (McLaughlin et al. 2017; O'Brien et al. 2017; Ryan et al. 2018).

El conocimiento de las bases genéticas de la ELA dio un paso fundamental con el descubrimiento de la expansión del hexanucleótido GGGGCC en la región no codificante del gen *C9ORF72* (DeJesus-Hernandez et al. 2011; Renton et al. 2011). Esta expansión sigue un patrón de herencia autosómico dominante con penetrancia incompleta y se caracteriza por su pleiotropía, habiéndose descrito multitud de fenotipos neurodegenerativos y psiquiátricos (Cooper-Knock et al. 2014). Actualmente constituye la causa genética más frecuente de ELA familiar y esporádica en nuestro ámbito (27.2% y 3.1% respectivamente en la población española)(García-Redondo et al. 2013), así como de DFT familiar (25%) y esporádica (5%), y del complejo ELA-DFT (van Blitterswijk et al. 2012).

Poco después del descubrimiento de la expansión en *C9ORF72* la generalización del uso de las técnicas de secuenciación masiva, ha permitido un avance exponencial en el conocimiento de las causas genéticas de la enfermedad, con el descubrimiento de varios genes nuevos cada año (Chia et al. 2018). Muchas de estas mutaciones (Strong et al. 2017), se han detectado también en pacientes con DFT, confirmando una superposición genética que ya se veía a nivel clínico-patológico. Las características de los genes causales de ELA más frecuentes en nuestro ámbito están recogidas en la Tabla 1. Actualmente (http://alsod.iop.kcl.ac.uk/) se han descrito mutaciones en más de 30 genes con herencia mendeliana (muchos también descritos en la DFT), aunque la mayoría de mutaciones pueden encontrarse también hasta un 10% de los pacientes aparentemente esporádicos. Es más, no existen diferencias relevantes en el fenotipo clínico, la anatomía patológica o la respuesta al tratamiento entre el global de las formas

familiares y las esporádicas. De esta manera, se ha difuminado de esta forma la clásica diferenciación entre formas esporádicas y familiares y actualmente parece imposible inferir una etiología genética en la ELA basada exclusivamente en los antecedentes familiares (Turner et al. 2017). Por otro lado, aunque la mayoría de los genes descritos segregan autosómico dominante, la penetrancia suele ser incompleta, siendo la presencia de co-mutaciones (mutaciones en dos genes causales de ELA) mayor de lo esperado por el azar (Cady et al. 2015). Esto dibuja un panorama cada vez más complejo donde la enfermedad podría tener una base oligogénica, de forma que varias mutaciones actuarían de forma concomitante, junto con determinados factores ambientales, modificando el riesgo de padecerla (Al-Chalabi et al. 2016a; Chiò et al. 2018). Conocer las bases genéticas de cada enfermo puede ser muy relevante a la hora de encontrar tratamientos eficaces ya que la respuesta a los mismos podría variar en función de su sustrato genético (van Eijk et al. 2017).

Gen	Locus	Herencia	Fenotipo	Frecuencia ELAf	Frecuencia ELAe
C9orf72	9p21.3-p.13.3	Dominante^	ELA, ELA-plus, ELA-DFT, DFT	25-50%	3-8%
SOD1	21q-22.1	Dominante*	ELA, AMP	20%	1%
TDP43 (oTARDP)	1p36.2	Dominante	ELA, ELA-DFT, DFT	1-5%	<1%
FUS	16p11.2	Dominante	ELA juvenil, ELA-DFT, DFT	1-5%	<1%

Tabla 1. Mutaciones más frecuentes en la esclerosis lateral amiotrófica y fenotipos más frecuentemente asociados.

C. BIOMARCADORES PARA EL DIAGNÓSTICO Y ESTRATIFICACIÓN DE LA ELA

La necesidad del desarrollo de biomarcadores en la ELA, se justifica por la baja sensibilidad y el retraso en el diagnóstico con los criterios clínicos actuales, por la dificultad actual de realizar predicciones pronósticas fiables y por la dificultad para cuantificar con precisión la pérdida neuronal que determina la progresión de la enfermedad. Así, se distinguen respectivamente tres tipos de biomarcadores según su

utilidad (Turner and Benatar 2015): biomarcadores diagnósticos, biomarcadores de progresión y biomarcadores pronósticos.

Los biomarcadores diagnósticos deben ser capaces de diferenciar la ELA de otras entidades que pueden simular una ELA con una elevada sensibilidad pero particularmente con elevada especificidad (dado el impacto de este diagnóstico). Además, idealmente, deben ser capaces de hacerlo en un momento temprano desde el inicio de los síntomas.

Los biomarcadores de progresión deben permitir monitorizar la evolución de la enfermedad sin verse influidos por factores ajenos a la misma. Idealmente deben ser más sensibles en la detección de cambios que las escalas de discapacidad, calidad de vida o en la exploración neurológica.

Los biomarcadores pronósticos deben ser capaces de predecir la evolución y pronóstico (en ELA, habitualmente se mide la supervivencia libre de traqueostomía) con una adecuada exactitud.

BIOMARCADORES BASADOS EN IMÁGENES DE RESONANCIA MAGNÉTICA

La resonancia magnética (RM) cerebral permite medir fundamentalmente la afectación del área motora y otras áreas cerebrales. Su amplia disponibilidad y limitado coste la convierten en una de las técnicas con mayor potencial en el campo de los biomarcadores. La combinación de varios parámetros de neuroimagen avanzada permite medir simultáneamente cambios estructurales, bioquímicos y funcionales en los sujetos mejorando su sensibilidad y especificidad en el diagnóstico de la ELA (Mazón et al. 2018). De todos los biomarcadores de RM cerebral utilizados con esta finalidad, vamos a destacar tres para los fines de este trabajo:

A. Medición de depósitos de hierro en córtex precentral

La aparición de un ribete hipointenso en secuencias basadas en T2 en la circunvolución precentral se describió hace más de 20 años en pacientes de ELA (Ishikawa et al. 1993), pero avances recientes en la técnica de resonancia como nuevas secuencias de susceptibilidad magnética (T2* o susceptibility-weighted, SW) o campos magnéticos más altos, han incrementado su interés como biomarcador. Estos ribetes parecen

indicar, en pacientes de ELA, la presencia de depósitos de hierro en forma de ferritina el interior de microglía activada circundante a las motoneuronas en degeneración en las capas profundas del córtex motor (Kwan et al. 2012; Adachi et al. 2015). Sin embargo, estas hipointensidades se pueden detectar también en sujetos sanos con el envejecimiento (Callaghan et al. 2014) y su significado, tanto en pacientes de ELA como en controles, permanece sin aclarar.

B. Medición de las hiperintensidades de sustancia blanca en el tracto corticoespinal

Estas hiperintensidades, detectadas particularmente en la secuencia FLAIR, se han descrito en pacientes de ELA y, en menor magnitud, en controles sanos. Podrían ser reflejo de la degeneración de dichos tractos, aunque correlacionan poco con los signos de motoneurona superior (Mazón et al. 2018).

C. Grosor cortical

El análisis cuantitativo y automatizado del grosor cortical permite estudiar la atrofia de sustancia gris punto por punto en todas las regiones cerebrales. Esta medición se ha mostrado proporcional al grado de afectación funcional y deterioro cognitivo, ha demostrado correlación con la progresión así como buena sensibilidad y especificidad para diferenciar fenotipos, genotipos y grupos pronósticos (Mazón et al. 2018).

BIOMARCADORES ECOGRÁFICOS

La ecografía cerebral es una técnica poco invasiva, cada vez más utilizada en el diagnóstico de enfermedades neurodegenerativas. Así, por ejemplo, se ha demostrado útil para el diagnóstico de la enfermedad de Parkinson, por la detección, de unas hiperintensidades en la sustancia negra que, supuestamente, se corresponden también con depósitos de hierro en esa localización (Berg et al. 2008). Estas hiperintensidades se pueden detectar también en pacientes de ELA, pero no parecen relacionarse con ninguna variable clínica, por lo que su significado resulta incierto (Fathinia et al. 2013). Adicionalmente, la ecografía cerebral permite medir el diámetro del tercer ventrículo, que parece ser un buen marcador de la atrofia cerebral global (Wollenweber et al. 2011). Esto puede servir tanto para el diagnóstico diferencial de los parkinsonismos (Berg et al. 2008), como para la detección del deterioro cognitivo en la esclerosis múltiple (Kallmann et al. 2004). Sin embargo, su papel como biomarcador en la ELA permanece sin aclarar.

Por otro lado, la ecografía muscular se ha propuesto como una técnica que puede aumentar la sensibilidad del electromiograma (EMG) en la detección de fasciculaciones, especialmente en la región bulbar (Misawa et al. 2011), facilitando el diagnóstico y permitiendo una monitorización de la afectación de motoneurona inferior de forma poco invasiva. Sin embargo, la aportación de la ecografía muscular al conocimiento fisiopatológico de las fasciculaciones no se ha estudiado.

2. JUSTIFICACIÓN

La ELA es una enfermedad muy heterogénea clínicamente lo que resulta en un importante retraso diagnóstico y en último término en la ausencia de tratamientos eficaces. Una adecuada caracterización clínica y genética de los pacientes es fundamental de cara a lograr entender las causas de la enfermedad en cada individuo y alcanzar un tratamiento individualizado. También los biomarcadores de imagen pueden ser una ayuda adicional para lograr un diagnóstico precoz y para caracterizar a los pacientes en subgrupos (endofenotipos) con una fisiopatología propia. Estos endofenotipos podrían tener una evolución diferente y una respuesta distinta a los tratamientos. Por tanto, una caracterización clínica, genética y de neuroimagen de los pacientes de ELA es básica para caminar hacia una medicina más personalizada.

Por lo que respecta a la caracterización clínica, hasta hace poco tiempo se consideraba que la ELA era una enfermedad exclusiva de las motoneuronas. Hoy sabemos que se afectan otras estructuras cerebrales y que esta extensión patológica es responsable de la aparición de otros síntomas como deterioro cognitivo o de comportamiento (Strong et al. 2017) o parkinsonismo (D'Ascenzo et al. 2012). Recientemente se ha descrito también la presencia de sintomatología urinaria en pacientes de ELA (Lopes de Carvalho et al. 2011; Nübling et al. 2014), aunque la causa de la misma es desconocida puesto que no existe afectación patológica del núcleo de Onuf. Caracterizar clínica y urodinámicamente a los pacientes con sintomatología urinaria, puede ayudar a determinar los mecanismos subyacentes.

Dentro de los biomarcadores de MNI, la principal limitación del EMG es que resulta una prueba molesta y que muestra una reducida sensibilidad para detectar fasciculaciones, particularmente en determinados grupos musculares en los que resulta difícil conseguir el reposo muscular (Misawa et al. 2011). En este sentido, la ecografía muscular puede ser una herramienta útil para estudiar las fasciculaciones y su relación con las características demográficas y clínicas de los pacientes.

En los últimos años se han producido importantes avances en la búsqueda de biomarcadores neurofisiológicos y de neuroimagen de MNS en la ELA (Huynh et al. 2016). Los biomarcadores de neuroimagen estructural pueden obtenerse tanto con RM como con ecografía cerebral. Los biomarcadores cualitativos de RM , que incluyen las hipointensidades en el área motora en secuencias de susceptibilidad magnética y las

hiperintensidades en los tractos corticoespinales en secuencias T2 y FLAIR, tienen la ventaja de su fácil traslación clínica (Mazón et al. 2018). También la ecografía permite obtener, de forma sencilla y con un bajo coste, imágenes de la sustancia negra y tercer ventrículo cerebral (Berg et al. 2008), que se han descrito alterados en la ELA (Fathinia et al. 2013). Sin embargo, el significado de exacto de todos estos biomarcadores en la ELA permanece sin aclarar, limitando su uso en la práctica clínica.

La caracterización genética de los pacientes de ELA es fundamental para estudiar la influencia del trasfondo genético en los síntomas y biomarcadores, ayudando a interpretar su significado y los mecanismos subyacentes.

Por último, es esencial conocer cómo la heterogeneidad clínica afecta al trayecto y retraso diagnóstico de los pacientes de ELA, para poder intervenir de forma adecuada acortando en lo posible el proceso diagnóstico y favoreciendo un inicio precoz del tratamiento.

3. OBJETIVOS

El objetivo general de la presente tesis es la caracterización clínico-genética de una cohorte de pacientes de ELA, así como evaluar la contribución de los biomarcadores de imagen en el diagnóstico, seguimiento y pronóstico de estos pacientes considerando su fenotipo y la presencia o no de mutaciones causales.

Los objetivos específicos son los siguientes:

1. Caracterizar los síntomas motores y no motores (cognitivos y urinarios) de una cohorte de pacientes de ELA.
2. Estudiar la presencia de mutaciones causales y su relación con los antecedentes familiares, incluyendo la esquizofrenia.
3. Evaluar la influencia de los factores clínicos y genéticos en el retraso diagnóstico y la calidad de vida de los pacientes.
4. Determinar el significado y la utilidad de los biomarcadores de imagen cerebral (RM y ecografía) en los pacientes de ELA.
5. Determinar la utilidad de la ecografía muscular para la detección y análisis de las fasciculaciones en pacientes de ELA.

4. METODOLOGÍA

Los pacientes fueron reclutados y valorados de forma prospectiva entre enero 2014 y diciembre de 2017 en consultas externas de neurología del Hospital Universitario y Politécnico la Fe. Se incluyó en el estudio a todos los pacientes que deseaban participar, que firmaron el consentimiento informado y que fueron diagnosticados de ELA o sus variantes incompletas. También se incluyeron, para determinados estudios, controles sanos o bien pacientes con otras enfermedades en las que se planteó en algún momento el diagnóstico de ELA dentro del diagnóstico diferencial (imitadores de ELA) y dieron su consentimiento.

D. RECOGIDA DE VARIABLES DEMOGRÁFICAS Y CLÍNICAS

En una base de datos Access creada ad-hoc se recogieron variables tanto de forma retrospectiva como prospectiva.

Las variables recogidas de forma prospectiva incluían variables demográficas como la fecha de nacimiento, sexo, tabaquismo e índice de masa corporal (IMC) y variables clínicas como la fecha de inicio de síntomas y de diagnóstico y la región y lateralidad de inicio de los síntomas.

La recogida retrospectiva de variables se realizó mediante la revisión de las notas médicas derivadas de cada atención, registradas en el sistema de información clínico-asistencial hospitalario (programa Orion Clinic) y el Sistema de Información de la Asistencia Ambulatoria de la Agencia Valenciana de Salud (SIA-Gaia), así como de los informes de otros centros no integrados en dichos sistemas, aportados por los propios pacientes. Las variables recogidas incluían: fecha y médico de primera consulta, especialistas visitados y fechas, pruebas realizadas, fechas de estudios electrofisiológicos, consultas en urgencias, diagnóstico emitido por el neurólogo, fecha de diagnóstico, fecha de inicio de riluzol.

E. CARACTERIZACIÓN CLÍNICA

FENOTIPADO

Los pacientes fueron divididos en tres fenotipos principales: ELA para aquellos que cumplían criterios diagnósticos de posible, probable con apoyo de laboratorio, probable o definitivo de El Escorial revisado (Brooks et al. 2000); AMP, en aquellos casos con

afectación exclusiva de motoneurona inferior y en el que otras causas se habían descartado de forma razonable (Garg et al. 2017); ELP, en aquellos casos con afectación exclusiva de motoneurona superior que cumplían los criterios previamente definidos (Gordon et al. 2006).

En los estudios en los que sólo se incluyeron pacientes con ELA, se diferenció entre: formas clásicas de ELA (ELAc), en aquellos pacientes con afectación clínica evidente de MNS y MNI; formas con afectación predominante de la MNI (ELA-MNI), incluyendo los fenotipos flail arm y flail leg (Al-Chalabi et al. 2016b); y formas con predominio de afectación de la MNS (ELA-MNS) según fue definida por Gordon et al. (Gordon et al. 2009).

VALORACIÓN MOTORA

Como hemos señalado, la ELA afecta predominantemente a las motoneuronas superior e inferior produciendo una debilidad progresiva. Por tanto, la exploración neuromuscular debe incluir la valoración de las motoneuronas superior e inferior. Para los fines de este estudio se utilizaron las siguientes escalas motoras:

a. ALSFRS-R: Es una escala ampliamente utilizada y validada que mide de 0-48 la discapacidad de los pacientes según la afectación de las distintas regiones corporales (bulbar, cervical, dorsal, lumbar) (Cedarbaum et al. 1999).
b. MRC: Es una escala ampliamente utilizada y validada que puntúa de 0-5 la fuerza en cada grupo muscular (Florence et al. 1992), siendo una medida indirecta del grado de afectación de la motoneurona inferior.
c. UMN score: Es una escala ampliamente utilizada y validada que contabiliza de 0-12 el número de reflejos patológicos, incluyendo: mentoniano, orbicularis oris, bicipital (2), tricipital (2), estiloradial (2), Hoffman (2), patelar (2), aquíleo (2), cutáneo-plantar (2).

VALORACIÓN COGNITIVA

En los pacientes se midió de forma sistemática la fluidez verbal con la letra P, con la que se calculó el índice de fluidez verbal (Strong et al. 2017). En aquellos pacientes anártricos, se midieron la serie de dígitos invertida y el *trail making test*. Para todas estas

escalas se utilizaron los puntos de corte ajustados para edad y nivel educativo establecidos en población española (Pena-Casanova et al. 2009; Tamayo et al. 2012). Para la valoración conductual se administró al paciente de ELA y su cuidador la versión española del cuestionario FRSBE (Caracuel et al. 2012), utilizando los *Z-score* de las puntuaciones "post-enfermedad".

El diagnóstico de deterioro cognitivo leve, deterioro leve de comportamiento y demencia frontotemporal, se realizó siguiendo los criterios diagnósticos actuales en la ELA (Strong et al. 2017).

VALORACIÓN URINARIA

En algunos pacientes se realizó una valoración sistemática de los síntomas urinarios (de urgencia, incontinencia y vaciado) mediante las versiones españolas de las siguientes escalas: International Consultation on Incontinence Questionnaire Short Form (ICIQ-SF), Overactive Bladder Awareness Tool (OAB-V8) and International Prostate Symptom Score (IPSS). Los síntomas se consideraron clínicamente significativos con las siguientes puntuaciones: IPSS>7, ICIQ-SF>0, or OAB-V8>8. A los pacientes con estas puntuaciones, se les ofreció la posibilidad de valoración urológica, incluyendo un estudio urodinámico.

F. CARACTERIZACIÓN GENÉTICA

Todos los pacientes fueron interrogados sobre sus antecedentes familiares de ELA, demencia u otras enfermedades neurodegenerativas. Los pacientes con antecedentes de ELA o sugerentes de demencia fronto-temporal que cumplían criterios de ELA familiar (Byrne et al. 2011), se categorizaron como formas familiares (ELAf), mientras que el resto se categorizaron como formas esporádicas (ELAe). En todos los pacientes (familiares y esporádicos) se estudió la expansión en *C9ORF72* mediante *repeat-primed* PCR y los casos positivos se confirmaron mediante southern blot. En los pacientes con ELAf en los que no se encontró expansión en *C9ORF72*, se secuenciaron los genes *SOD1*, *TARDBP* y *FUS* en busca de mutaciones puntuales. Tras el análisis genético, se reclasificó a los pacientes esporádicos portadores de mutaciones causales como ELAf.

G. DETERMINACIONES ANALÍTICAS

Se recogieron los niveles de creatinina al diagnóstico y en los pacientes que consintieron, se realizó una analítica para determinar los niveles de ferritina mediante inmuno-turbidimetría.

H. BIOMARCADORES DE RESONANCIA MAGNÉTICA

Los estudios de resonancia magnética se realizaron con un scanner de 3 Tesla (Signa HDxt, GE Healthcare, Milwaukee, USA). El protocolo de resonancia incluía una secuencia 3DT1 axial (TR, 6.6 ms; TE, 2.8 ms; TI, 400; FOV, 220 x 220 mm2; matrix, 256 x 256 mm2; flip angle, 12º; voxel size 1 x 0.94 x 0.94 mm3); una secuencia SW obtenida a partir de una secuencia 3D multi- eco gradiente T2* (TR = 43 ms; 9 echoes with centre TE around 26 ms; TE = 15.1-35.8 ms with 5.1 echo spacing; FOV = 220 × 220 mm; matrix = 256 × 256 mm; slice thickness = 2 mm, flip angle = 15°; bandwidth = 62.5 Hz/px); y una secuencia FLAIR se obtenida a partir de una secuencia 2D FSE T2 (TR = 11000 ms; TE = 150 ms; TI = 2250 ms; FOV = 220 × 220 mm; matrix = 256 × 256 mm; slice thickness = 2 mm; intersection gap =1 mm; flip angle = 90°; bandwidth = 41.67 Hz/px).

La secuencia 3DT1 se utilizó para el cálculo del volúmenes corticales siguiendo la segmentación automática de Freesurfer, versión 5.2 (http://surfer.nmr.mgh.harvard.edu/).

Los cambios de intensidad en las secuencias SW y FLAIR fueron evaluadas de forma semicuantitativa por dos neurorradiólogos ciegos al diagnóstico. La hipointensidad en las imágenes SW se evaluó en la parte posterior de la corteza motora en los hemisferios izquierdo y derecho. La corteza motora se subdividió en tres subregiones, que correspondían a la representación cortical de miembros inferiores, miembros superiores y musculatura bulbar, en cada hemisferio (Figura 2). La región del miembro superior se identificó utilizando la omega invertida como hito anatómico. Para seleccionar los miembros inferiores y las regiones bulbar, se utilizaron mapas somatotópicos basados en RM funcional y un atlas neuroanatómico. La intensidad del cambio de señal se puntuó de la siguiente manera: 0, intensidad normal; 1, intensidad de señal ligeramente hipointensa, similar al cuerpo calloso; 2, marcadamente hipointensa, señal similar a las venas subependimarias. Finalmente, sumando las puntuaciones parciales de cada sub-región se calculó un score global. La hiperintensidad en FLAIR se midió en el tracto

corticoespinal y en la sustancia blanca subcortical. De nuevo la intensidad de señal se cuantificó en ambas regiones de la siguiente manera: 0, intensidad normal; 1, intensidad de señal ligeramente hiperintensa, similar a la corteza; 2, marcadamente hiperintensa, señal más intensa que la corteza.

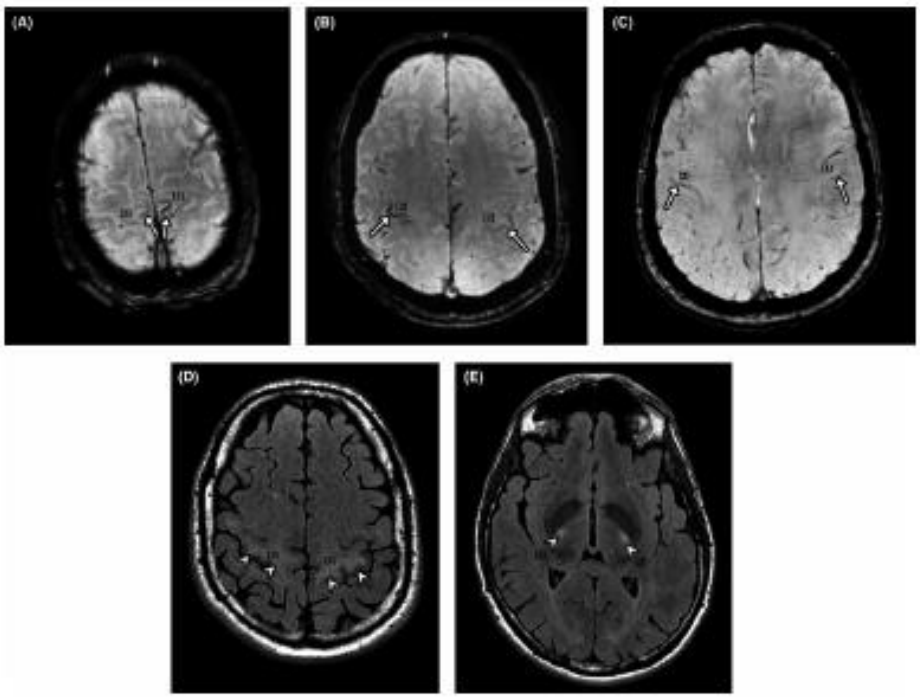

Figura 2. Localización de los cambios de intensidad de señal en pacientes de ELA. Extraída de Vázquez et al. 2018d.

I. BIOMARCADORES ECOGRÁFICOS

ECOGRAFÍA CEREBRAL

Un neurólogo con experiencia en ecografía y ciego a las características clínicas, realizó el estudio ecográfico con un equipo Toshiba Aplio XG (Tokyo, Japan 2008) equipado con un transdcutor de 2.5 MHz, obteniendo imágenes en modo B a través de la ventana acústica temporal bilateral. En estas imágenes se midió el diámetro del tercer ventrículo en corte transversal y el área de ecogenicidad de ambas sustancias negras siguiendo descripciones previas (Berg et al. 2008).

ECOGRAFÍA MUSCULAR

Un neurólogo con experiencia en ecografía y ciego a las características clínicas, realizó el estudio ecográfico con un Toshiba Aplio XG (Tokyo, Japan 2008) equipado con un

transductor de 7.2–14 MHz. Todos los parámeteros, como ganancia (80 dB), compensación, profundidad, amplitude, frecuencia (13 MHz), compresión y foco se mantuvieron constantes durante el estudio. Con el paciente en decúbito supino y posición relajada, se examinaron diez músculos que representaban las cuatro regiones anatómicas (bulbar, cervical, torácia y lumbar): lengua, cricotiroideo, trapecio, bíceps, tríceps, abductor corto del pulgar, recto abdominal, recto femoral, tibial anterior y gastrocnemio medial. Los músculos se exploraron bilateralmente salvo la lengua, por lo que en cada paciente se estudiaron un total de 19 músculos. Cada músculo se observó durante 30 segundos y se contabilizaron las fasciculaciones siguiendo la metodología previamente descrita (Misawa et al. 2011). Se obtuvieron además imágenes de cada músculo examinado y otro investigador ciego midió posteriormente el grosor muscular.

J. ANÁLISIS ESTADÍSTICO

Los análisis estadísticos se realizaron con el apoyo de la Unidad de Bioestadística del Instituto de Investigación Sanitaria La Fe utilizando el software R y el paquete clickR.

De forma resumida, para la descripción de los datos se utilizaron la media, mediana, desviación estándar y rango intercuartil para las variables cuantitativas y las frecuencias absolutas y relativas para las variables cualitativas. Además se representaron gráficamente los datos y sus relaciones utilizando *boxplots* y *heat maps*.

Para los análisis inferenciales se utilizaron correlaciones de Spearman y modelos multivariantes (regresiones logísticas, lineales, binomiales negativas mixtas…). Cuando la complejidad de los modelos lo requería, se utilizó la estadística bayesiana.

K. COMITÉ DE BIOÉTICA Y CONFIDENCIALIDAD

El estudio se realizó siguiendo las directrices de la declaración de Helsinki y contó con la aprobación del Comité Ético de Investigación Clínica del Hospital Universitario y Politécnico La Fe (número de proyecto 2013/0332, fecha de aprobación 5 de noviembre de 2013). Todos los participantes firmaron un consentimiento informado.

El tratamiento, la comunicación y a cesión de los datos de carácter personal de todos los sujetos participantes, se ajustó a lo dispuesto en la Ley Orgánica 15/1999, del 13 de diciembre, de protección de datos de carácter personal. La responsable del registro de

los datos en la Agencia Española de Protección de Datos fue la Conselleria de Sanitat. Las muestras de sangre utilizadas en el estudio fueron procesadas y conservadas en el Biobanco La Fe en cumplimiento con la ley orgánica de protección de datos.

5. RESULTADOS

En el estudio participaron un total 261 pacientes de ELA (incluyendo pacientes con atrofia muscular progresiva y esclerosis lateral primaria), 45 controles sanos y 28 pacientes con patologías que imitaban una ELA. No todos los sujetos participaron en todas las partes del estudio, dependiendo de la población objeto en cada trabajo, de las condiciones clínicas de los pacientes y de la voluntad de participar de cada sujeto. En este apartado se resumen los resultados de los trabajos directamente relacionados con el proyecto de caracterización clínica, genética y de neuroimagen de esta cohorte de pacientes y en los que el autor de la presente tesis doctoral actúa como autor principal. La tabla 2 resume las características demográficas, clínicas y genéticas de los pacientes que han participado en las investigaciones relacionadas con esta tesis doctoral, así como su participación en los diversos subestudios.

	N	Edad (años) Media (SD)	Sexo (varón) %	Fenotipo %	Espinal %	ELAf %	Mutaciones
Cohorte Global	261	62,1 (11,3)	57,5 %	ELA (87,7 %) ELP (5 %) AMP (7,3 %)	73,6 %	13,8 %	18 *C9ORF72* 9 *SOD1* 1 *FUS* 1 *TARDBP*
Subestudio síntomas urinarios	55	62,4 (10,9)	58.2 %	ELA (67 %) ELP (15 %) AMP (18 %)	83,6 %	5,4 %	2 *C9ORF72*
Subestudio retraso diagnóstico	143	61,7 (12)	57,3 %	ELA (100 %)	67,8 %	9,8 %	7 *C9ORF72* 3 *SOD1* 1 *FUS* 1 *TARDBP*
Subestudio RM cerebral	102	61,2 (12,1)	63,7 %	ELA (100 %)	81,4 %	9,8 %	9 *C9ORF72* 1 *SOD1*
Subestudio ecografía cerebral	117	62,3 (12,1)	59 %	ELA (77,8 %) ELP (8,5 %) AMP (13,7 %)	76,1 %	15,3 %	10 *C9ORF72* 6 *SOD1* 1 *FUS*
Subestudio ecografía muscular	44	65,4 (10,5)	54,5 %	ELA (100 %)	72,7%	15,9 %	4 *C9ORF72* 2 *SOD1*

Tabla 2. Resumen de las características demográficas, clínicas y genéticas de los pacientes participantes en los distintos subestudios.

A. CARACTERIZACIÓN CLÍNICA Y GENÉTICA

Todos los pacientes fueron caracterizados clínicamente y en 247 de ellos se realizaron los análisis genéticos pertinentes. Tanto las variables clínicas como genéticas, se utilizaron posteriormente para los estudios de biomarcadores, pero dieron también lugar a tres publicaciones adicionales.

En dos de ellas describíamos la presencia de sintomatología urinaria en 55 pacientes de ELA. Hasta ahora considerados síntomas marginales, encontramos que el 41.7% de los pacientes de ELA participantes presentaban sintomatología urinaria, siendo la intensidad de los mismos moderada (Arlandis et al. 2016). En 10 de ellos se realizó un estudio urodinámico siendo la presencia de disfunción vesico-esfinteriana el hallazgo más frecuente. Estos síntomas se presentaban con la misma frecuencia independientemente de la edad, el fenotipo, la afectación cognitiva, el estadio de enfermedad y la presencia o no de antecedentes familiares, aunque parecían ser menos frecuentes en mujeres y en el análisis de supervivencia la presencia de sintomatología urinaria parecía asociarse a peor pronóstico (Vázquez-Costa et al. 2017a).

En la tercera publicación (Vázquez-Costa et al. 2015), describíamos las características clínicas y de neuroimagen de dos hermanas portadoras de la expansión *C9ORF72* con expresiones clínicas diversas: una padecía ELA y la otra esquizofrenia. En esta publicación mostrábamos la divergencia endofenotípica y analizábamos, con datos de la literatura, un posible papel causal de la expansión *C9ORF72* en trastornos del desarrollo en general y en la esquizofrenia en particular.

B. ANÁLISIS DEL TRAYECTO DIAGNÓSTICO

En este trabajo (Vázquez-Costa et al. 2018c), se analizó el trayecto diagnóstico de 143 pacientes con ELA clásica (57% varones, 68% de inicio espinal). El 86% de ellos habían sido estudiados en centros públicos y un 14% en privados. El retraso diagnóstico medio fue de 13,1 meses (mediana 11.7). El paciente tardó de media 7,9 meses en llegar al neurólogo y este, 5,2 meses más en diagnosticarlo. En la mitad de los pacientes se realizaron pruebas innecesarias y más de un estudio electrofisiológico para llegar al diagnóstico. El retraso diagnóstico fue mayor en los casos espinales ($p = 0,008$),

atribuible a los pacientes cuyos síntomas se iniciaron en miembros inferiores, pero sin diferencias entre el sistema público y privado (p = 0,897).

C. BIOMARCADORES DE IMAGEN CEREBRAL

Como resultado de los estudios de biomarcadores de imagen cerebral se publicaron tres trabajos: uno analizando biomarcadores de RM cerebral y dos de ecografía cerebral.

RM CEREBRAL

En este estudio (Vázquez-Costa et al. 2018d) se incluyeron 102 pacientes con ELA y 48 controles (28 imitadores de ELA y 20 controles sanos). En los controles, los depósitos de hierro en el área motora, pero no las hiperintensidades en la sustancia blanca, se asociaron con la edad. En los pacientes con ELA, ambos biomarcadores se asociaron al grado de afectación de MNS y al inicio bulbar. La intensidad y extensión de los depósitos de hierro en las diferentes regiones del motor homúnculo (extremidades inferiores, extremidades superiores y bulbar) se relacionaron con el sitio de inicio de los síntomas. No se encontraron diferencias en ambos biomarcadores entre los pacientes genéticos y esporádicos.

ECOGRAFÍA CEREBRAL

Para los estudios de biomarcadores de ecografía cerebral, se reclutaron un total de 108 pacientes de ELA (incluyendo AMP y ELP) y 25 controles. En estos estudios se incluyeron además cohortes históricas de pacientes con trastornos del movimiento (fundamentalmente enfermedad de Parkinson) y de controles sin enfermedades neurodegenerativas.

En el estudio del tercer ventrículo (Vázquez-Costa et al. 2018b), encontramos que los pacientes presentaban diámetros mayores que los controles sanos. El tamaño del tercer ventrículo se asoció a la edad, el inicio espinal y la presencia de deterioro cognitivo o de comportamiento, pero no a la presencia de mutaciones, el fenotipo o la discapacidad. Además pudimos demostrar que un aumento del tercer ventrículo en pacientes de ELA se relacionaba con una disminución de la sustancia gris subcortical, pero no con otras estructuras cerebrales.

En el estudio de la sustancia negra (Vázquez-Costa et al. 2017b), demostramos que los pacientes de ELA presentan un área de hiperecogenicidad mayor que la de controles sin enfermedades neurodegenerativas, pero similar a la enfermedad de Parkinson y otros trastornos del movimiento. De forma interesante, encontramos que la sustancia negra izquierda presentaba un área de hiperecogenicidad mayor que la derecha y que los varones presentaban hiperecogenicidades mayores que las mujeres. Finalmente demostramos que los pacientes portadores de mutaciones presentaban hiperecogenicidades mayores que los no portadores, siendo ésta la única variable clínica que explicaba las diferencias encontradas entre pacientes de ELA.

D. BIOMARCADORES DE IMAGEN MUSCULAR

Se realizó ecografía en 835 músculos de 44 pacientes de ELA de diagnóstico reciente (< 90 días). La ecografía detectó fasciculaciones con más frecuencia que el EMG. Las fasciculaciones en pacientes de ELA eran generalizadas, especialmente en aquellos de inicio en miembros superiores, y afectando a los músculos proximales particularmente en la región cervical (bíceps). El número de fasciculaciones se asoció de manera inversa con la discapacidad (ALSFR-R) y el IMC y directamente con la pérdida de IMC y la afectación de MNS. Nuestro modelo estadístico sugería que las fasciculaciones aumentan con la degeneración inicial de la MNI, alcanzan su punto máximo cuando el músculo se torna de leve a moderadamente débil, disminuyendo luego con el aumento de la debilidad y atrofia muscular.

6. DISCUSIÓN

El presente trabajo realiza aportaciones relevantes al conocimiento de la ELA, tanto en la descripción de su heterogeneidad clínica y genética, como en el papel de distintos biomarcadores en el conocimiento de la enfermedad.

En primer lugar, confirmamos estudios previos (Lopes de Carvalho et al. 2011; Nübling et al. 2014) que mostraban una alta prevalencia de sintomatología urinaria en pacientes de ELA. En los artículos anexos se demuestra que dichos síntomas son atribuibles en la mayoría de los casos a una disfunción vesico-esfinteriana y no a la movilidad reducida (Arlandis et al. 2016). Se muestra además que pueden aparecer tanto en formas genéticas como esporádicas y en todos los fenotipos y fases de la enfermedad, si bien su aparición podría determinar un peor pronóstico (Vázquez-Costa et al. 2017a). Por lo tanto, los síntomas urinarios son frecuentes en pacientes de ELA y deben ser tenidos en cuenta en la práctica clínica.

En segundo lugar, se describe una familia portadora de la mutación *C9ORF72* en la que una hermana presenta una ELA y otra, una esquizofrenia. Recientemente se ha descrito un aumento de prevalencia de esquizofrenia en familiares de pacientes de ELA (O'Brien et al. 2017). Esta asociación es mayor en familias portadoras de *C9ORF72* (O'Brien et al. 2017; Devenney et al. 2018) apoyando la hipótesis de una base genética compartida entre estas dos patologías (McLaughlin et al. 2017). La familia descrita en la publicación anexa (Vázquez-Costa et al. 2015), muestra la heterogeneidad fenotípica y de neuroimagen de dos portadoras de *C9ORF72* y es representativa de la pleiotropía propia de esta mutación (Rohrer et al. 2015b, 2015a).

En tercer lugar, se muestra que el retraso y trayecto diagnóstico de los pacientes de ELA en nuestro medio es independiente del modelo de atención sanitaria y similar a los de países de nuestro entorno (Vázquez-Costa et al. 2018c). Conocer el trayecto diagnóstico de los pacientes de ELA es fundamental para poder desarrollar estrategias de diagnóstico precoz. Nuestros datos sugieren que el retraso acumulado tiene diversas causas como son, el tiempo que tarda en consultar el paciente por los síntomas iniciales, el tiempo que tarda el paciente en llegar al neurólogo y el tiempo que tarda el neurólogo en llegar al diagnóstico (Vázquez-Costa et al. 2018c). Acortar uno sólo de esos tiempos sin influir en el resto (como ocurre por ejemplo en la sanidad privada, donde el paciente tiene acceso directo al neurólogo) podría no ser suficiente para reducir

significativamente el retraso diagnóstico, como un estudio previo sugería (Mitchell et al. 2010).

Por último, se analiza el significado de diversos biomarcadores de imagen. Las hipointensidades en el área motora y las hiperintensidades en los tractos corticoespinales eran dos biomarcadores descritos hace tiempo (Hecht et al. 2002), pero se conocía poco sobre su significado y utilidad. En una amplia cohorte demostramos que ambos son marcadores de degeneración de la MNS, si bien las primeras son más específicas que las segundas (Vázquez-Costa et al. 2018d). Es más, la localización de las hipointensidades se corresponde bastante bien con la localización de la clínica en cada paciente, por lo que podría ser un buen biomarcador diagnóstico y de progresión (Vázquez-Costa et al. 2018d). En contraposición a estos hallazgos, observamos que la hiperecogenicidad de la sustancia negra, que se había descrito en pacientes de ELA (Fathinia et al. 2013), no presenta correlato clínico, si no que viene determinada por el trasfondo genético de los pacientes (Vázquez-Costa et al. 2017b). Este hallazgo es relevante porque pone de manifiesto que esta hiperecogenicidad, presente en gran variedad de enfermedades neurodegenerativas (Berg et al. 2008), no es un marcador de degeneración si no de vulnerabilidad neuronal, como se había propuesto previamente (Berg 2011). Por otro lado, se confirma en una publicación que el diámetro del tercer ventrículo medido por ecografía se encuentra aumentado en pacientes de ELA y que este marcador refleja el grado de atrofia subcortical que ocurre con la edad y se asocia a la presencia de deterioro cognitivo (Vázquez-Costa et al. 2018b). Este trabajo abre la puerta al uso de este parámetro, fácil y rápido de medir, como un predictor de la presencia o aparición de deterioro cognitivo en un paciente. Finalmente, haciendo uso de la ecografía muscular, investigamos el significado de las fasciculaciones en pacientes de ELA. Hasta ahora, éstas se habían relacionado con la presencia de hiperexcitabilidad (Geevasinga et al. 2016). Nosotros demostramos por primera vez su asociación con la pérdida de peso que tiene lugar en las primeras fases de la enfermedad, probablemente atribuible a la pérdida de masa grasa por exceso de consumo energético (Vázquez-Costa et al. 2018a). Describimos además, gracias a complejos modelos estadísticos, la dinámica de aparición de fasciculaciones y los factores que influyen en su incremento y decremento con la evolución de la enfermedad (Vázquez-Costa et al. 2018a). Estos

hallazgos son relevantes para entender un fenómeno que es característico de la ELA y que parece tener un valor pronóstico (Shimizu et al. 2014). Futuros estudios deberán determinar si atajar precozmente la pérdida de peso asociada a fasciculaciones permite neutralizar esta asociación pronóstica.

7. CONCLUSIONES

De la presente tesis doctoral se pueden extraer las siguientes conclusiones:

1. La ELA es una enfermedad clínicamente heterogénea en la que la debilidad es el síntoma más prominente pero en la que son también frecuentes otros síntomas como los cognitivos y conductuales (Vázquez-Costa et al. 2018b) y los urinarios (Arlandis et al. 2016; Vázquez-Costa et al. 2017a). Puesto que los síntomas urinarios son tratables, se debería preguntar sistemáticamente a los pacientes de ELA por su presencia.
2. La heterogeneidad clínica de la ELA es, probablemente en parte, responsable del retraso diagnóstico, que en nuestro medio es similar a lo descrito en otros países y que no parece depender del sistema sanitario (Vázquez-Costa et al. 2018c).
3. Los errores diagnósticos del neurólogo son frecuentes y en parte atribuibles a una mala orientación o interpretación del estudio electrofisiológico (Vázquez-Costa et al. 2018c). De este modo, la formación específica del neurólogo y neurofisiólogo general y la derivación precoz a centros de referencia podrían ayudar a reducir la demora.
4. La ELA es una enfermedad genéticamente heterogénea. En nuestro medio la causa genética más frecuente de ELA es la expansión en *C9ORF72* (Vázquez-Costa et al. 2017b, 2018b, 2018d), que podría causar también trastornos del neurodesarrollo y esquizofrenia (Vázquez-Costa et al. 2015).
5. La ELA afecta a estructuras cerebrales más allá de la corteza motora, produciendo atrofia cerebral subcortical que es, al menos en parte, responsable de la aparición de deterioro cognitivo y/o cambios del comportamiento (Vázquez-Costa et al. 2018b). En este sentido el diámetro del tercer ventrículo podría ser un marcador sencillo para detectar y/o monitorizar la aparición de deterioro cognitivo en pacientes de ELA (Vázquez-Costa et al. 2018b).
6. La ELA se asocia a cambios de intensidad de señal tanto en la sustancia negra como en el área motora, detectables por ecografía y RM respectivamente, que podrían estar determinados por el depósito de determinados metales. Sin embargo, estos cambios parecen tener significados distintos según su localización. Por un lado los depósitos de hierro en el área motora y las hiperintensidades de sustancia blanca son un marcador de degeneración

neuronal y por tanto son mayores en los fenotipos con mayor afectación de la MNS (ELA clásica, ELP e inicio bulbar) frente a los fenotipos con menor afectación (AMP e inicio espinal), pero no se influyen por la presencia de mutaciones (Vázquez-Costa et al. 2018d). Por otro lado, la hiperecogenicidad de sustancia negra, cuya causa es menos conocida, no se asocia a variables clínicas en la ELA si no a variables constitucionales y genéticas y por lo tanto parece ser más un marcador de vulnerabilidad neuronal que de neurodegeneración (Vázquez-Costa et al. 2017b).

7. La ecografía muscular es un método sensible, sencillo y poco invasivo para la detección de fasciculaciones en la ELA (Vázquez-Costa et al. 2018a).
8. La afectación de MNS y MNI causa las fasciculaciones en la ELA y éstas a su vez contribuyen a la pérdida de peso que tiene lugar previamente al diagnóstico (Vázquez-Costa et al. 2018a).
9. Las fasciculaciones son particularmente frecuentes en músculos proximales y de miembros superiores, siendo el grado variable de degeneración de la MNI el principal determinante de las mismas (Vázquez-Costa et al. 2018a).

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9. ANEXO: TRABAJOS PUBLICADOS

A. VÁZQUEZ-COSTA JF, BELTRÁN E, SOPENA P, SABATER A, CARDONA F, VILCHEZ JJ, ET AL. CLINICAL AND NEUROIMAGING CHARACTERIZATION OF TWO C9ORF72-POSITIVE SIBLINGS WITH AMYOTROPHIC LATERAL SCLEROSIS AND SCHIZOPHRENIA. AMYOTROPH LATERAL SCLER FRONTOTEMPORAL DEGENER. 2015;17(3–4):297–300.

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Clinical and neuroimaging characterization of two *C9ORF72* positive siblings with amyotrophic lateral sclerosis and schizophrenia.

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Keywords: Amyotrophic Lateral Sclerosis, Schizophrenia, *C9ORF72*, Frontotemporal Dementia.

INTRODUCTION

C9ORF72 expansion is the main genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) and has also been found in a wide spectrum of other neurodegenerative diseases (1).

Schizophrenia is a neurodevelopmental mental disorder associated with a combination of rare nonspecific variants with a high genotypic relative risk and common genetic variation in hundreds of different alleles (2).

Recently, an increased occurrence of certain psychiatric conditions (including schizophrenia) in kindreds with the *C9ORF72* repeat expansion has been shown (3), suggesting a causal role of this mutation.

We present a family carrying the *C9ORF72* mutation that co-segregates with ALS and schizophrenia.

CASE 1

A 53 years old woman without relevant personal history came to our clinic after a 1-year history of weakness and atrophy in left hand and leg. Her father had been diagnosed with rapidly progressive dementia at the age of 75 and died within 4 years (Fig. 1). Two brothers of her father had been diagnosed and reportedly died of ALS .She had two sisters, one diagnosed with schizophrenia and the other institutionalized in a nursing home because of severe mental retardation, which was present from early childhood and was attributed to a traumatic brain injury at age one. Neurological and electrophysiological examination confirmed the involvement of upper and lower motor neuron in two regions. An extensive neuropsychological battery showed a significant impairment of language and executive functions. MRI showed widespread (mainly frontal) cortical and subcortical atrophy (Fig. 2A). FDG-PET analysis revealed a widespread frontotemporal hypometabolism that was significant in frontotemporal structures (Fig. 3A). She was diagnosed with probable ALS according to the El Escorial criteria, with mild cognitive impairment. A repeat primed PCR showed a hexanucleotide repeat expansion in *C9ORF72*, that was confirmed with Southern blot (>4000 repeats).

5 of her asymptomatic relatives were screened for the variant. The expanded variant was detected in her sister, who carried a diagnosis of schizophrenia (Fig. 1).

CASE 2

She is a 49 years old woman with a mild mental retardation who was diagnosed with paranoid schizophrenia at age 18, following symptoms of auditory hallucinations. She was successfully treated with neuroleptic drugs, although she continued to have negative symptoms. Her neurological examination was unremarkable and her intelligent quotient (IQ) score was 58, with significant impairment in language, verbal and visual memory and visuospatial functions and relative preservation of executive functions. Her MRI showed mild enlargement of the lateral ventricles, especially of the occipital horns, suggesting subcortical (mainly occipital) atrophy (Fig. 2B), without white matter changes in T2 weighted images. FDG-PET showed hypometabolism in bilateral occipital lobes and some frontotemporal sulcus (Fig. 3B).

DISCUSSION

This work characterizes clinically and radiologically two siblings with ALS and schizophrenia carrying the *C9ORF72* expansion, that appears to co-segregate with disease.

C9ORF72-positive ALS patients are clinically characterized by a high frequency of comorbid cognitive and behavioural impairment, and radiologically by widespread cortical frontotemporal and subcortical atrophy and hypometabolism (1). Intriguingly, a recent study in presymptomatic *C9ORF72* carriers has shown early widespread (mainly posterior) atrophy up to 25 years and multi-domain cognitive and behavioural impairment up to 20 years before expected disease onset (4), suggesting early developmental brain changes.

Schizophrenia is a heterogeneous neurodevelopmental disorder, influenced by genetic factors and recently found to be overrepresented in *C9ORF72*-positive pedigrees (3). Although several studies have searched for this mutation in over a thousand schizophrenia patients, overall only three patients tested positive (5). Since clinical and neuroimaging features in schizophrenia can overlap with those of FTD, especially in those carrying the C9ORF72 expansion (6,7), the increased prevalence of schizophrenia

in *C9ORF72*-pedigrees could actually reflect misdiagnosed FTD cases. However, case 2 is compatible with a classical paranoid schizophrenia evolving to residual schizophrenia, excluding the possibility of misdiagnosis. Interestingly, neuropsychological and neuroimaging findings were neither those of typical schizophrenia nor those of FTD. Specifically, executive functions were preserved, whereas MRI and FDG-PET showed atrophy and hypometabolism mainly in bilateral occipital lobes.

Neuroimaging findings in case 1 are those typically found in *C9ORF72*-positive ALS patients, whereas in case 2 seem rather to overlap with those found in presymptomatic carriers.

Interestingly, histological studies in *C9ORF72* expansion carriers show deposits of the transcriptional dipeptide repeat (DPR) products of C9ORF72 in cerebellum, hippocampus and occipital cortex prior to TDP-43 deposits that are preferentially found in frontal lobes (8,9). Recently, a case report raised the question of whether DPRs can lead to early childhood intellectual disability (9) and increasing evidence suggests a clinical continuum between some neurodevelopmental psychiatric disorders and neurodegenerative diseases (3,6,10). Since *C9ORF72* has been linked to some neurodevelopmental disorders (1,3), a causative role could be hypothesized (9). However, this remains unproved, giving the low epidemiological evidence and the lack of known molecular mechanisms.

Further studies are warranted to define, if possible, an imaging endophenotype of *C9ORF72*-positive schizophrenia patients and to untangle the role of this mutation in neurodevelopmental disorders.

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Ethics Approval: The ethical committee for biomedical research of Hospital La Fe has approved this study. Written consent was obtained from mentioned individuals to allow the publication of this case report. Samples were processed , preserved and delivered by the Biobank La Fe, licensed as required by RD 1716/2011, of 18 November, by which the basic requirements for authorization and operation of biobanks are established for biomedical research and treatment of biological samples of human origin (Ref: PT13 / 0010/ 0026).

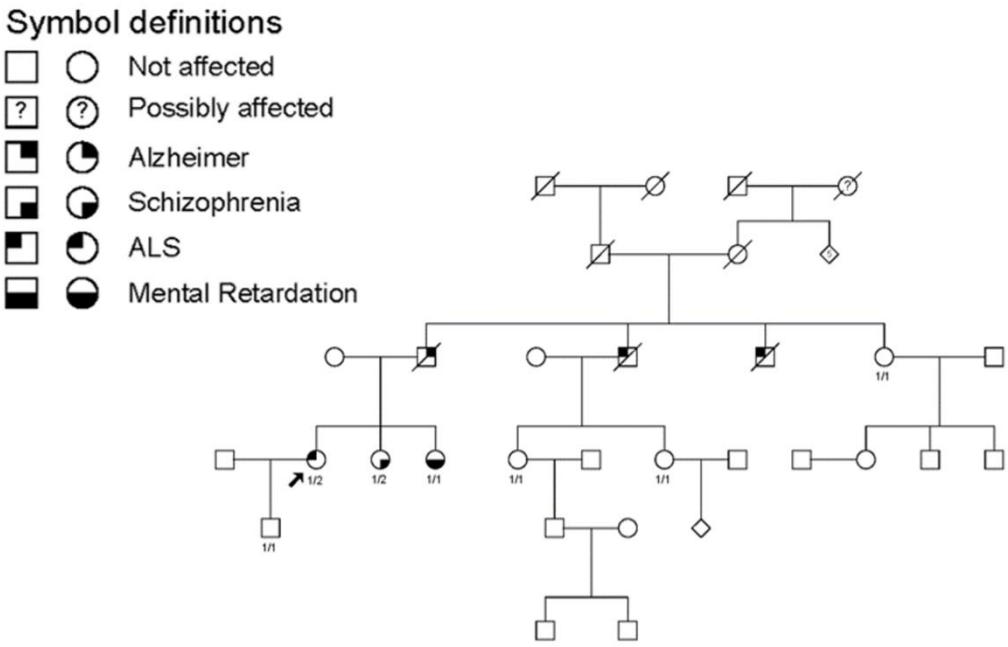

Figure 1. Family kindred

Filled symbols are those subjects with neurological or psychiatric conditions. The proband is indicated by an arrow. The top number is the number of the individual; the bottom number represents the C9orf72 status (1/1: no expansion; 1/2: expansion in heterozygosis).

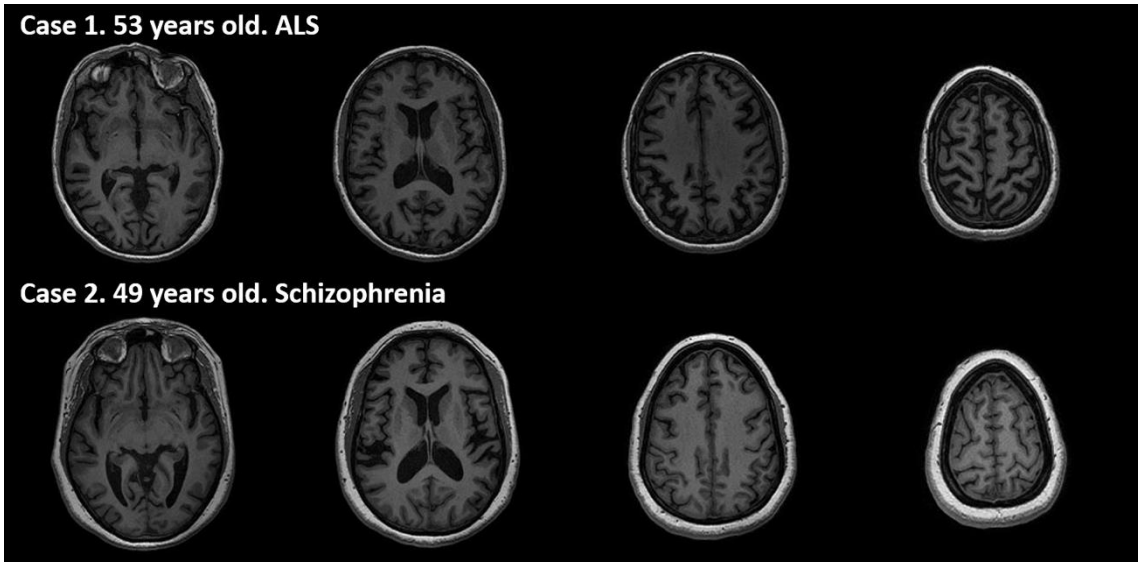

Figure 2. MRI findings.

A) 3D T1 weighted axial MRI of the brain of the ALS patient (53 years old) showing widespread cortical and subcortical atrophy with moderate ventricular enlargement. Larger frontoparietal atrophy in the ALS patient than in the schizophrenia patient can be seen in the top sections.

B) 3D T1 weighted axial MRI of the brain of the schizophrenia patient (49 years old) revealing wider enlargement of the occipital horns of the lateral ventricles than patient A, suggesting larger occipital white matter atrophy. Similar enlargement in the body as well as in the frontal and temporal ventricular horns is shown, indicating similar subcortical atrophy. However, less sulci enlargement in the cortex suggest less cortical atrophy.

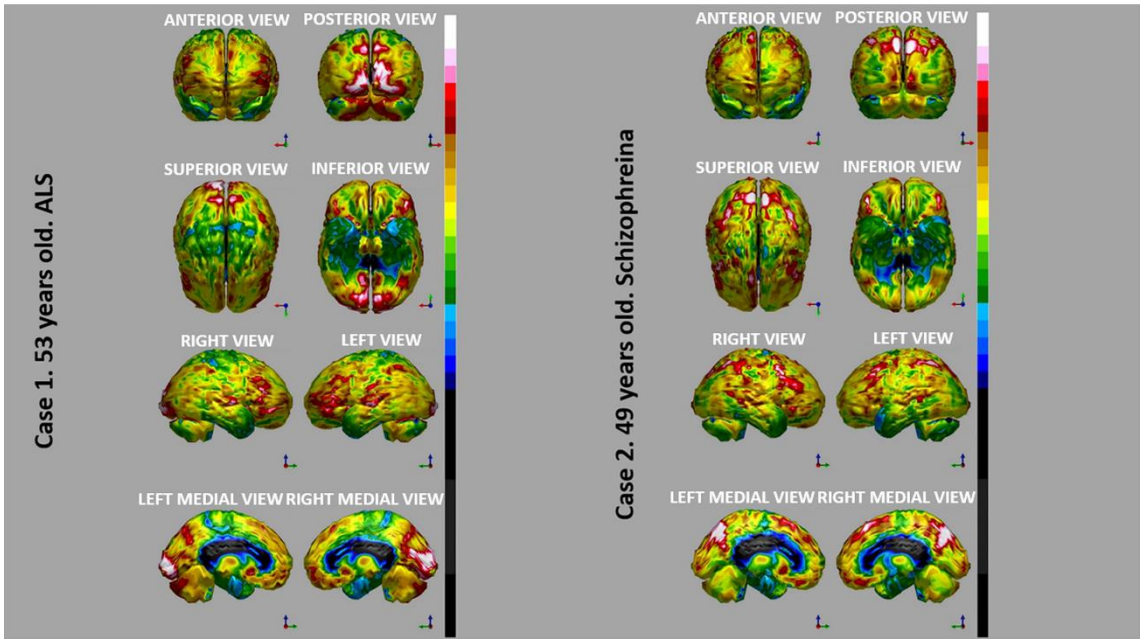

Figure 3. A Fludeoxyglucose PET (FDG-PET) was performed and analysed using the 3.5 software version of the NeuroQ, which facilitates an automated regional assessment by average values of pixel regions of interest and standardized to a desired standard brain structure quantifications. Patient values were compared with those from a database of control population with an uptake below or above 1.65 standard deviations considered as abnormal. A 3D stereotaxis representation of the brain was done by using OASIS software.

Brain FDG-PET of the ALS patient (53 years old) showing frontotemporoparietal hypometabolism, especially in bilateral precentral gyrus, frontal inferior gyrus, cingulate gyrus and anterior medial and lateral temporal cortex.
Brain FDG-PET of the schizophrenia patient (49 years old) revealing mild hypometabolism in bilateral occipital lobes, anterior medial and lateral temporal gyrus, as well as in bilateral inferior frontal gyrus. Left thalamus and bilateral precentral gyrus and, compared to the ALS patient, cerebellum, also appear to be mild hypometabolic, although these areas did not reach significance in statistical analysis.

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TITLE

Urodynamic findings in amyotrophic lateral sclerosis patients with lower urinary tract symptoms: results from a pilot study

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ABSTRACT

Aims: To determine LUTS prevalence and urodynamic findings in ALS patients treated in our hospital.

Methods: Cross-sectional and descriptive study on a cohort of ALS patients. Validated questionnaires (ICIQ-SF, IPSS and OAB-V8) were self-administered in order to evaluate the presence of LUTS. Symptoms were classified as clinically significant (csLUTS), if any of following scores, IPSS > 7, ICIQ-SF > 0 or OAB-V8 ≥ 8, were present. Urodynamic study was offered to csLUTS patients. Physical examination and prostate ultrasound were also performed.

Results: 55 of 79 (70%) ALS patients accepted to participate in the study. Only 24/55 (43.6%) patients met criteria for csLUTS and 13 patients reported urgency urinary incontinence (26.3%). Most of csLUTS patients complained of mixed symptoms (82.6%). QoL measured by IPSS was 2.1±1.5, 20% scoring as mostly dissatisfied or unhappy. Average QoL ICIQ-SF scoring was 3.17±3, 33% complained of moderate to severe bother. Ten of 24 (41.7%) csLUTS patients consented to UDS. The most frequent finding was detrusor overactivity with obstruction due to non-relaxing external sphincter (5 patients) or bladder neck (2 patients). Two patients showed normal bladder filling but non-relaxing external sphincter during voiding. UDS was normal in one patient.

Conclusions: In this small pilot study we found a high prevalence of csLUTS in ALS which are mainly related to a combination of voiding and storage symptoms. In most patients, symptoms are caused by overactive detrusor combined with non-relaxing sphincter. Severity of symptoms and impact in QoL is only moderate but in a subset of patients can be considerable.

KEY WORDS

Amyotrophic Lateral Sclerosis; Urodynamics; Lower Urinary Tract Symptoms.

ABBREVIATIONS AND ACRONYMS

LUTS = Lower Urinary Tract Symptoms

ALS = Amyotrophic Lateral Sclerosis

ICIQ-SF = International Consultation on Incontinence Questionnaire Short Form

IPSS = International Prostate Symptom Score

OAB-V8 = Overactive Bladder Awareness Tool

ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale Revised

csLUTS = clinically significant LUTS

QoL = Quality of Life

UMN = Upper Motor Neuron

LMN = Lower Motor Neuron

PLS = Primary Lateral Sclerosis

PMA = Progressive Muscular Atrophy

UI = Urinary Incontinence

UUI = Urgency Urinary Incontinence

UDS = Urodynamic Study

EMG = Electromyography

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that typically involves upper (UMN) and lower motor neurons (LMN). This disease is clinically characterized by a progressive weakening of the muscles, that starts either in the bulbar region (bulbar ALS) or in the limbs (spinal ALS) and spreads leading to death of respiratory failure in 3-5 years (1).

Three main phenotypes can be differentiate according to the presence or absence of clinical or electrophysiological signs of UMN and LMN (1): classic ALS, when both UMN and LMN signs can be elicited; primary lateral sclerosis (PLS), when only UMN impairment is found; and progressive muscular atrophy (PMA), when only LMN impairment is found. Although weakness is the most characteristic feature of ALS, cognitive, sensory and dysautonomic symptoms are not infrequent (2). These symptoms are the result of the spreading of the disease to different neuronal networks other than pyramidal tracts and supports the view of ALS as a multisystem disorder (2). Consequently, lower urinary tract symptoms (LUTS) have been reported in ALS patients although its prevalence differs widely between studies ranging from 4 to 40% (3–5). Moreover, underlying causes and pathophysiologic mechanisms have not been properly studied. Since Onuf's nucleus has been shown to remain relatively spared in ALS (6), clinical guidelines and textbooks still consider LUTS as an atypical feature in ALS or a result of reduced mobility in advanced stages of the disease (7). However, other structures involved in micturition such as the brainstem reticular formation or the spinal intermediolateral column have been shown to be affected in ALS (3,8).

In order to determine LUTS prevalence in ALS patients, we conducted a cross-sectional study on a cohort of patients treated at the ALS Unit of University Hospital La Fe. Moreover, urodynamic studies were performed in those symptomatic ALS patients to determine the underlying bladder dysfunction leading to LUTS.

MATERIAL AND METHODS

Subjects and definitions

We conducted a cross-sectional and descriptive study to determine LUTS prevalence in an ALS cohort of patients. Patients were recruited among those followed at the ALS Unit at La Fe University Hospital between May and November 2014. Included in the study were those who gave written consent and met diagnostic criteria of ALS (possible, probable or definitive according to El Escorial criteria) (9), PMA (10) or PLS (11). At recruitment, urinary symptoms scales were administered for self-completion to patients who consented to participate. Urinary incontinence (UI) and urge urinary incontinence (UUI) were defined according to the 2002 International Continence Society (12). Those patients who reported significant urinary symptoms and consented were referred to the urologist for urodynamic evaluation (see below).

The study was approved by local ethics committee and all the procedures followed the declaration of Helsinki. All participants gave written informed consent.

Studied variables

Age, gender, history of diabetes and alcohol consumption and time and place of symptom onset were recorded for all participants. Heavy drinkers were considered those consuming 15 drinks or more per week for men and eight for women, according to the definition of the Center for Disease Control and Prevention in Atlanta (www.cdc.gov). Concomitant medication was registered from clinical records.

The disability level was measured with the Amyotrophic Lateral Sclerosis Functional Rating Scale Revised score (ALSFRS-R score) (13).

Presence of LUTS was evaluated using validated questionnaires in Spanish language: International Consultation on Incontinence Questionnaire Short Form (ICIQ-SF),

Overactive Bladder Awareness Tool (OAB-V8) and International Prostate Symptom Score (IPSS).

ICIQ-SF (14) is a short questionnaire to evaluate the presence of urinary incontinence including frequency, severity and bother. Scores ranges from 0 to 21, where 0 means complete urinary continence. Any positive score reveals UI.

OAB-V8 (15) is an eight item questionnaire used for screening OAB in primary care population. Scores ranges from 0 to 40. A positive screen score ≥ 8 yield an odds ratio of 95.7 for having probable overactive bladder (16).

IPSS is a 7 item questionnaire evaluating storage and voiding symptoms in male patients, with an additional question concerning quality of life (QOL-IPSS). Scoring ranges from 0 (not at all) to 5 (always) for each item, with a global scoring from 0 to 35. A severity symptomatology categorization has been established, ranging from mild (0-7), moderate (8-19) to severe symptoms (20-35). Scores of QOL-IPSS ranges from 0 (delighted) to 6 (terrible) (17). Storage (questions 2, 4 and 7) and voiding symptoms (questions 1,3,5 and 6) were also evaluated separately and patients where categorized into three groups: those with only storage symptoms, those with only voiding and those with mixed symptoms.

All three questionnaires were self-administered to ALS patients. Symptoms were classified as clinically significant (csLUTS) with any of the following scores: IPSS > 7, ICIQ-SF > 0 or OAB-V8 ≥ 8.

Patients with csLUTS were offered to perform a urodynamic study (UDS) at the Urology Department. A complete UDS was done including free uroflowmetry, post-void residual measurement, filling cistomanometry and pressure flow study with perineal surface electromyography, under the International Continence Society recommendations of good urodynamic practice [Schaeffer]. Free flowmetry was always performed before invasive urodynamics in order to minimize influence of catheters on urine flow and eventually artifacts. A dipstick test was routinely performed before each UDS in order

to exclude any concomitant urinary tract infection. UDS studies were done using a MMS Solar^{TM} system, with v8.19 software measurement. Pelvic and genital examination, bladder and prostate ultrasound and plasma PSA levels (male patients) were also performed. Prostate volume was assessed by trans-abdominal ultrasonography using the ellipsoid formula (cc).

Statistics.

Data were summarized by mean, standard deviation, median and range in the case of continuous variables and by relative and absolute frequencies in the case of categorical variables. Differences between groups were analyzed using either Student's t-test or Wilcoxon test for normal and non-normal quantitative variables respectively. Chi-square analyses were performed for categorical data. P values < 0.05 were considered statistically significant. All statistical analyses were performed using R software (version 3.2.0).

RESULTS

Prevalence and characteristics of LUTS

From May to November 2014, 55 of 79 ALS patients followed at the ALS Unit and were offered to participate. 55 (70%) of them gave written consent and were included in our study. A summary of demographic and clinical characteristics of the study cohort can be found in Table 1 and data of the non-included patients in Supplementary Table 1. All patients completed ICIQ-SF questionnaires, although one OAB-V8 and five IPSS questionnaires were missing. Twenty-four ALS patients (43.6%) met criteria for csLUTS and more than half of them (54.2%, 13/24), that is 26.3% of the 55 studied patients, reported UI. Clinical and demographical characteristics of csLUTS and UI patients were similar to those of non-csLUTS and non-UI patients respectively (Table 2), although a trend to more males was found among csLUTS patients (70.8% vs 48.4%, p=0.094).

Severity of LUTS in csLUTS patients was only moderate. 23 of 24 csLUTS patients fulfilled the IPSS questionnaire and 17 of them (73.9%) scored >7. Most of these 17 complained of both storage and voiding symptoms (13/17, 76.5%). Only one patient (1/17, 5.9%) presented pure storage symptoms and three (3/17, 17.6%) pure voiding symptoms. Details of the scores can be found in Table 3. The impact of urinary symptoms on quality of life as measured with QoL-IPSS was mild to moderate (mean score 2.1±1.5), with only 4 out of 20 patients (20%) scoring as mostly dissatisfied or unhappy (4-5 points) and 11 out of 20 (55%) scoring from delighted to mostly satisfied (0-2 points). All UI patients met criteria for UUI and the average bother of UI among incontinent patients, measured by ICIQ-SF questionnaire was 3,17 ± 3 (0-8) and only 33% of them complained of moderate to severe bother of UI (>5 points on ICIQ-L).

Urodynamic studies

Ten of the 24 (41.7%) csLUTS patients consented to do UDS. Clinical and demographical characteristics of those patients were similar to those who didn't consent (Table 4). However, impact of LUTS on quality of life was significantly higher in patients who consented and a trend to more frequent UUI and higher ICIQ-SF and IPSS scores was found. Nine of ten (90%) patients show some abnormality in the urodynamic study and in five of them findings were considered severe: high pressure involuntary detrusor contractions (most of them over 70 cmH_2O) and high detrusor pressure in voiding (Table V). The most frequent finding was detrusor overactivity with obstruction due to non-relaxing external sphincter (5 patients) or bladder neck (2 patients). Two additional patients showed normal bladder filling but non-relaxing external sphincter during voiding. Drugs that could have an impact in lower urinary tract function were recorded in each patient, but none of them was completely responsible of the urodynamic findings. In male patients prostate enlargement was excluded by ultrasound (mean prostate volume 25,5 cc ± 6,4, 14-34). Bladder, prostate and kidney ultrasound were performed in every patient with csLUTS after UDS, and no abnormalities were found. Urinalysis and urine cultures were normal in all csLTUS patients.

Table V summarizes urodynamic findings, prostate volume and drugs taken by each patient. In one of the studied patients, the UDS was normal. This patient reported UUI with both storage and voiding symptoms and she had high scores in ICIQ-SF (15), OABV-8 (7), IPSS (14). However, symptoms cannot be attributed to reduced mobility, since she had been diagnosed with bulbar ALS and strength on upper and lower limbs was still present at the time of the examination. No concomitant urinary tract infection, and no pelvic abnormalities were found.

DISCUSSION

Previously considered to be atypical features in ALS, recent studies have shown a high prevalence on cognitive, sensitive and dysautonomic symptoms. LUTS have been scarcely studied on ALS patients and differences in methodology make it difficult to draw conclusions on frequency of symptoms.

We performed a comprehensive study of LUTS using ICIQ-SF, OAB-V8 and IPSS Spanish validated questionnaires. We chose these questionnaires because are commonly used in our daily practice as screening tools. We analyzed separately voiding and storage symptoms. Cut-off points were defined for each questionnaire according to validation studies and patients scoring above those cut-off points were considered as symptomatic. Following this methodology, we found a LUTS prevalence of 43.6%, with 82.6% of them reporting both voiding and storage symptoms, and a UI prevalence of 26.3% (all of them classified as UUI). These results are similar to those published before. For example, Lopes de Carvalho et al reported a prevalence of urinary symptoms of 40% in ALS patients according to El Escorial criteria, but they do not clarify whether PLS phenotype was included or not, nor which questionnaires and methodology were used to asses LUTS (5). Both voiding (“incomplete emptying”) and storage (“nocturia”, “frequency”) symptoms were frequently found (24 and 22% respectively) but UI was seldom reported (5.5%). However, a comparison to normal population or a measure of the severity of those urinary symptoms were lacking. Nübling et al assessed LUTS in ALS, PLS and PMA patients using the ICIQ-SF and the Urinary Distress Inventory (UDI-6) questionnaires and compared the results to those of healthy German population. They

found a significantly higher prevalence of UI (33%) in patients than in controls: 73% of UI were UUI (4), but prevalence of other urinary symptoms was not reported. Our UI prevalence ratio is slightly lower (26%), but 100% suffered from UUI.

In our study, impact on quality of life of LUTS or incontinence measured by QoL-IPSS and ICIQ-SF was only moderate, although one third of incontinent patients reported significant impact on quality of life. This relatively reduced impact is not surprising considering the high global disability burden of ALS and probably explains that LUTS have gone unnoticed for so many years.

Causes and mechanisms underlying LUTS in ALS patients have been scarcely studied and to the best of our knowledge, no single UDS in ALS patients has been reported before. Therefore, since Onuf's nucleus is relatively preserved in the disease (8), LUTS were largely (but without any evidence) attributed to reduced mobility. However, in our study csLUTS patients did not differ in disease disability or duration from non-csLUTS patients, suggesting that urinary symptoms are not related with severity of the disease, as previously reported (4). Moreover, we did not find differences in other causes of dysautonomia such as diabetes or alcohol abuse between both groups.

In order to identify the underlying bladder dysfunction we aimed to perform UDS to csLUTS patients. As far as we know, this is the first paper about urodynamic findings in ALS patients. Only ten of them (41.7%) consented, and they reported, as expected, slightly more severe urinary symptoms and impact on quality of life than those who did not consent. However, demographical and clinical characteristics were similar, so we think that results of UDS can be extrapolated to the csLUTS patient population. Urodynamic findings suggested neurogenic bladder in 9/10 (90%) patients and were considered mild in four of them and severe in five. Findings cannot be attributed to diabetes mellitus or alcohol abuse, since most of these patients (7 of 9) were neither diabetic nor heavy drinkers. Moreover, drugs taken by patients were registered, and none of them could explain completely urodynamic findings. Patients taking benzodiazepines and baclofen may have lower urethral resistance due to external and periurethral sphincter relaxation. Pregabalin has been described as a potential detrusor

muscle relaxing drug in rats (18) and increases mean voided volume in overactive bladder women (19). Only serotonin-norepinephrine reuptake inhibitors may play a role increasing urethral resistance through a theoretically increase of the tone of external and periurethral sphincter, but in clinical practice this fact is very rarely observed.

We cannot define a clear urodynamic pattern for ALS patients, due to the low number of patients that accept UDS. Nevertheless, a trend to overactive detrusor combined with high urethral resistance secondary to non relaxing external sphincter or bladder neck was found in 7/10 (70%) of patients. As we don't usually perform videourodynamics, a non-relaxing bladder neck is diagnosed by exclusion, when an obstructive pattern (high detrusor pressure at Qmax with low Qmax) is found in absence of augmented activity of external sphincter on EMG. This finding would correspond to a supra-spinal lesion on Madersbacher neurogenic bladder classification (20). Both brainstem reticular formation and spinal intermediolateral nucleus are involved in ALS (3,6,8). These lesions might justify this bladder-sphincter function pattern.

Only one patient showed a normal UDS although she reported significant LUTS. In this case symptoms were not caused by reduced mobility, concomitant urinary tract infection or pelvic abnormalities. Therefore, a false negative study cannot be excluded.

Our study is small, mainly descriptive and exploratory, and further studies are necessary to confirm the existence of a typical urodynamic pattern in ALS. One drawback of this study is that not all participants underwent an UDS and probably only those with more severe LUTS accepted it. This could result in selection bias, but it was not possible include all the cohort of patients. Moreover, we didn't perform video-urodynamic studies. We usually reserve this examination to more complex neurogenic patients at risk of upper urinary tract deterioration, or failed lower urinary tract surgeries. Our usual UDS include surface EMG, without X-ray imaging, and therefore it may be a problem for an exact detrusor sphincter dyssynergia diagnosis in some cases.

More studies are needed to explore the relationship between LUTS, bladder dysfunction and clinical characteristics of ALS patients, in order to reveal the underlying mechanisms of UUI.

CONCLUSIONS

Prevalence of clinically significant lower urinary symptoms in ALS is high and due to a combination of voiding and storage symptoms. In most patients, those symptoms are caused by overactive detrusor combined with non-relaxing sphincter. Severity of symptoms and impact on quality of life is only moderate but on a subset of patients it can be considerable. ALS patients should be asked for LUTS and when they are clinically significant, UDS and appropriate treatment should be offered.

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TABLES

Table I: Demographic and clinical characteristics of ALS population

Total *n* (%) from 79 patients	55 (70%)
Phenotype	ALS 37 (67%) PLS 8 (15%) PMA 10 (18%)
Age (years) *Mean (SD)* *Median (range)*	 62.4 (10.9) 62.8 (39.2 – 83.8)
Sex (male) *n* (%)	 32 (58.2%)
Diabetes mellitus *n* (%)	 9 (16,4%)
Heavy drinkers *n* (%)	 3 (5.5%)
Time from onset (months) *Mean (SD)* *Median (range)*	 46.3 (57.1) 30.8 (5.5 – 294)
Symptoms onset **Bulbar *n* (%)** **Spinal *n* (%)**	 9 (16.4%) 46 (83.6%)
ALSFRS-R *Mean (SD)* *Median (range)*	 31.1 (8.9) 33 (13 – 46)
ICIQ-SF, *n*=55 *Mean (SD)* *Median (range)*	 2.3 (4.8) 0 (0-18)
OAB-V8, *n*=54 *Mean (SD)* *Median (range)*	 6.5 (7.7) 4 (0- 35)
IPSS, *n*=50 *Mean (SD)* *Median (range)*	 5.8 (6.9) 3 (0-24)
csLUTS *n* (%)	 24 (43.6%)

ALS = amyotrophic lateral sclerosis; PLS = primary lateral sclerosis; PMA = progressive muscular atrophy; ALSFRS-R = revised ALS Functional Rating Scale; ICIQ-SF = International Consultation on Incontinence Questionnaire Short Form; OAB-V8 = Eight Item Overactive Bladder Awareness Tool; IPSS = International Prostate Symptom Score; csLUTS = clinically significant lower urinary tract symptoms.

Table II: Demographic and clinical characteristics of ALS patients with or without csLUTS and UI

ALS Patients	**csLUTS**	**Non-csLUTS**	***p***	**Incontinent**	**Continent**	***p***
Total n (%) from 55 patients	24 (43.6%)	31 (56.4%)		13 (26.3%)	42 (73.7%)	
Age (years) *Mean*	63.7	61.4	0.435	63.7	62	0.505
Sex (male) *n* (%)	17 (70.8%)	15 (48.4%)	0.094	9 (69.2%)	23 (54.8%)	0.355
Diabetes Mellitus *n* (%)	6/24 (25%)	3/31 (9.7%)	0.128	3/13 (23.1%)	6/42 (14.3%)	0.454
Heavy drinkers *n* (%)	1/24 (4.2%)	2/31 (6.2%)	0.711	1/13 (7.7%)	2/42 (4.8%)	0.684
Time from onset (months) *Median*	31.5	30.8	0.755	35.3	28.8	0.837
Symptoms onset Bulbar n (%) Spinal n (%)	 4 (16.7%) 20 (83.3%)	 5 (16.1%) 26 (83.9%)	0.957	 2 (15.4%) 11 (84.6%)	 7 (16.7%) 35 (83.3%)	0.913
ALSFRS-R *Mean*	30.8	31.3	0.846	31	31.1	0.964

ALS = Amyotrophic Lateral Sclerosis; ; csLUTS = clinically significant lower urinary tract symptoms.

Table III: Questionnaire scoring of csLUTS patients

csLUTS patients (n = 24)	**Mean (SD)**	**Median (range)**	**Clinically significant** ICIQ-SF >0 OAB-V8 ≥8 IPSS >7 QoL-IPSS >3
ICIQ-SF	4.4 (5.3)	3 (0-18)	13/24 (54.2%)
OAB-V8	12.1 (7.8)	10 (0-35)	18/24 (75%)
IPSS (*n*=23) • **IPSS storage** • **IPSS voiding**	11 (12.3) 5.2 (3.8) 5.7 (5.1)	4 (0-14) 4 (0-14) 5 (0-15)	17/23 (73.9%)
QoL-IPSS (*n*=20)	2.1 (1.5)	2 (0-5)	4/20 (20%)

csLUTS = clinically significant lower urinary tract symptoms. ; ICIQ-SF = International Consultation on Incontinence Questionnaire Short Form; OAB-V8 = Eight Item Overactive Bladder Awareness Tool; IPSS = International Prostate Symptom Score

Table IV: Clinical characteristics of patients with and without UDS

csLUTS Patients	**UDS**	**Non-UDS**	***p***
Total n (%) from 24 patients	10 (41.7%)	14 (58.3%)	
Age (years) *Mean*	61.5	65.2	0.368
Sex (male) *n* (%)	9/10 (90%)	9/14 (64.3%)	0.1515
Diabetes Mellitus *n* (%)	2/10 (20%)	1/14 (7%)	0.35
Heavy drinkers n (%)	1/10 (10%)	0/14 (0%)	0.223
Time from onset (months) *Mean*	28.26	66.93	0.136
Symptoms onset Bulbar n (%)	2/10 (20%)	2/14 (14.3%)	0.711
ALSFRS-R *Mean*	33	29.3	0.355
ICIQ-SF *Median*	6	0	0.06
UUI *n* (%)	7/10 (70%)	6/14 (42.9%)	0.188
OAB-V8 *Median*	16.5	9.5	0.088
IPSS (*n*=23) • IPSS storage • IPSS voiding *Median*	10 5 5	9.5 3.5 3.5	0.465 0.75 0.445
QoL-IPSS (*n*=20) *Median*	3	1	0.033*

Table V: Urodynamic findings in csLUTS patients

Case	**Gender**	**Symptoms**	**Prostate volume** (cc)	**Free Uroflowmetry** (Qmax/vol/pvr)	**Detrusor**	**DetPIC** (cmH_2O)	**Vol 1stIC** (mL)	**MCC** (mL)	**Urethra**	**Pdet Qmax** (cmH_2O)	**Qmax** (mL/s)	**PVR** (mL)	**Severity of UDS findings**	**Drugs**
1	Male	UUI	24	13/180/40	Normoactive	-	-	410	Non-relaxing external sphincter	46	12	25	Mild	escitalopram
2	Male	UUI and mixed LUTS	21	15/150/20	Overactive	26	91	360	Non-relaxing bladder neck	55	6	50	Mild	pregabalin
3	Male	UUI	34	5/140/110	Overactive	100	40	125	Non-relaxing bladder neck	97	6	0	Severe	-
4	Female	UUI and mixed LUTS	-	26/590/0	Normoactive	-	-	440	Normoactive	30	10	0	Normal	Amitriptyline diazepam, venlafaxine
5	Male	Voiding symptoms	25	14/160/50	Overactive	73	15	206	Non-relaxing external sphincter	72	8	0	Severe	-
6	Male	UUI and mixed LUTS	26	5/60/70	Overactive	80	160	305	Non-relaxing external sphincter	124	4	190	Severe	lorazepam, zolpidem

7	Male	UII and mixed LUTS	34	20/250/60	Overactive	25	400	480	Non-relaxing external sphincter	49	9	30	Mild	-
8	Male	Mixed LUTS	14	11/460/20	Normoactive	-	-	500	Non-relaxing external sphincter	48	2	400	Mild	baclofen
9	Male	Mixed LUTS	30	5/70/0	Overactive	46	18	190	Non-relaxing external sphincter	66	6	0	Severe	diazepam
10	Male	UII and mixed LUTS	22	9/130/400	Overactive	83	260	540	Non-relaxing external sphincter	62	6	410	Severe	venlafaxine

UUI = urgency urinary incontinence; LUTS = lower urinary tract symptoms; DetPIC = Detrusor pressure at involuntary contraction; Vol1stIC = volume at first involuntary contraction; MCC = maximum cystometric capacity; PdetQmax = Detrusor pressure at maximum flow during voiding; Qmax = maximum flow rate during pressure-flow study; PVR = Post Void Residual after pressure-flow study.

C. **VÁZQUEZ-COSTA JF, ARLANDIS S, HERVAS D, MARTÍNEZ-CUENCA E, CARDONA F, PÉREZ-TUR J, BROSETA E, SEVILLA T. CLINICAL PROFILE OF MOTOR NEURON DISEASE PATIENTS WITH LOWER URINARY TRACT SYMPTOMS AND NEUROGENIC BLADDER. J NEUROL SCI. 2017;378:130–6.**

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Clinical profile of motor neuron disease patients with lower urinary tract symptoms and neurogenic bladder

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Clinical profile of amyotrophic lateral sclerosis patients with lower urinary tract symptoms and neurogenic bladder

Introduction

Lower urinary tract symptoms (LUTS) are frequent in motor neuron disease (MND) patients, but clinical factors related to them are unknown. We describe differences in LUTS among MND phenotypes and their relationship with other clinical characteristics, including prognosis.

Methods

For this study, we collected clinical data of a previously published cohort of patients diagnosed with classical amyotrophic lateral sclerosis (cALS), progressive muscular atrophy (PMA) or primary lateral sclerosis (PLS) with and without LUTS. Familial history was recorded and the C9ORF72 expansion was analysed in the entire cohort. Patients were followed-up for survival until August 2016.

Results

Fifty-five ALS patients (37 cALS, 10 PMA and 8 PLS) were recruited. Twenty-four reported LUTS and neurogenic bladder (NB) could be demonstrated in nine of them. LUTS were not influenced by age, phenotype, disability, cognitive or behavioral impairment, or disease progression, but female sex appeared to be a protective factor (OR = 0.39, p = 0.06). Neither family history nor the C9ORF72 expansion were linked to LUTS or NB. In the multivariate analysis, patients reporting LUTS early in the disease course tended to show poorer survival.

Conclusions

In this study, LUTS appear to be more frequent in male MND patients, but are not related to age, clinical or genetic characteristics. When reported early, LUTS could be a sign of rapid disease spread and poor prognosis.

Further prospective longitudinal and neuroimaging studies are warranted to confirm this hypothesis.

Keywords: motor neuron disease; amyotrophic lateral sclerosis; progressive muscular atrophy; primary lateral sclerosis; lower urinary tract symptoms; neurogenic bladder; urodynamics.

Introduction

The term motor neuron disease (MND) encompass a group of neurodegenerative diseases typically involving upper (UMN) and/or lower motor neurons (LMN), leading to progressive weakness (1). Three different phenotypes can be distinguished according to the degree of involvement of UMN and LMN (1,2): classical ALS (cALS), primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA) (1). Although weakness is the most characteristic feature of MND, cognitive, sensory and dysautonomic symptoms are more frequent than previously thought (2). Extra-motor symptoms in MND are the result of the disease spreading beyond motor neurons (2–4), which can have prognosis or treatment implications. For example, cognitive impairment associates to faster progression and reduced survival in MND patients and autonomic impairment has been related to sudden death (5–8).

Urine storage and micturition depend on the coordinated interplay of several neuronal structures and systems. When some of these structures become impaired, different lower urinary tract symptoms (LUTS) such as frequency, urgency, nocturia and urinary incontinence (UI) can appear, a condition which is usually termed as neurogenic bladder (9,10). Three main urodynamic patterns can be distinguished (9,10). Overactive bladder (OAB) is usually the result of involuntary detrusor contractions during bladder filling and is characterized by storage symptoms. Detrusor-sphincter dyssynergia (DSD) is the result of a combination of detrusor overactivity and the absence of urethral sphincter inhibition during micturition. Clinically, it is characterized by both storage and voiding symptoms. Finally, hypocontractile detrusor results in urinary retention with predominantly voiding symptoms.

LUTS were previously thought to be infrequent features in MND (11,12). When occurred, they were largely attributed to reduced mobility, since Onuf's nucleus remains relatively spared in the disease (13). However, we and others have shown that LUTS are reported by around 40% of MND patients and that UI is found in up to 30% of them (14–16). Moreover, we have shown that these urinary symptoms are secondary to neurogenic bladder and therefore attributable to disease pathogenesis (16), although the underlying mechanisms remain unknown. We think that characterization of LUTS among MND phenotypes and its correlation with clinical characteristics can

help us to understand the underlying pathophysiology. This could be important, since the different mechanisms leading to UI can have diverse impact on treatment and/or prognosis, as mentioned above.

This study aimed to describe, in a previously published cohort of patients (16), differences in LUTS among MND phenotypes and their relationship with other clinical characteristics, including prognosis.

Patients and methods

Subjects and definitions

This study is an extension of a previously published cross sectional descriptive study (16) that aimed to determine LUTS prevalence in a MND cohort of patients. Patients were recruited among those visiting our ALS Unit between May and November 2014. Patients are routinely evaluated by the same neurologist (JVC) and demographical and clinical data are prospectively recorded in a database. We included in the study patients who gave written consent and were diagnosed with cALS, PMA or PLS. cALS patients met El Escorial revised criteria of possible, probable or definitive ALS (17). PMA was defined as a progressive isolated impairment of LMN at least in two regions (18) and PLS as a progressive isolated impairment of UMN in at least one region different from the lumbar region (19). Patients were followed-up for survival until August 2016.

The study was approved by the ethics committee for biomedical research of Hospital La Fe. All participants gave written informed consent.

Studied variables

Age, gender, history of diabetes, duration of motor symptoms, region of motor symptoms onset, and concomitant medication were recorded for all participants.

At recruitment, patients were assessed for: disability (ALSFRS-R) (20); muscle strength in limbs and neck using the Medical Research Council (MRC) rating scale (21) with a composite score with a normal value of 130; UMN impairment (UMN score), for a maximum of 16; and dysexecutive and behavioural impairment (a more detailed description of the neuropsychological assessment can be found as a supplemental file). The disease progression rate was calculated using the following formula: (48 – ALSFRS-

R score at the assessment visit)/time from symptoms onset in months, where 48 is the maximum ALSFRS-R score. Frontotemporal dementia (FTD) was diagnosed according to current criteria (22).

Family history of MND or FTD on first and second degree relatives was systematically recorded and, when present, patients were categorized as familial MND (fMND). All patients were screened for *C9ORF72* with repeat-primed PCR (23) and in those fMND not carrying a *C9ORF72* expansion, *SOD1*, *TARDBP* and *FUS* genes were subsequently analysed by Sanger sequencing.

The presence of LUTS were evaluated using Spanish self-completion validated questionnaires for screening of UI (International Consultation on Incontinence Questionnaire Short Form, ICIQ-SF), OAB (Overactive Bladder Awareness Tool, OAB-V8) and storage and voiding symptoms (International Prostate Symptom Score, IPSS). LUTS were considered as clinically significant (csLUTS) with any of the following scores (16): ICIQ-SF > 0, OAB-V8 ≥ 8 or IPSS > 7.

Patients with csLUTS were offered to perform functional studies at the Urology Department (16). Patients with csLUTS and abnormal urodynamic findings not related to pathology of the lower urinary tract were considered as having neurogenic bladder. According to these results, patients were classified as having neurogenic bladder (NB+) and not having neurogenic bladder (NB-). Patients not reporting csLUTS were considered NB-. Patients with csLUTS but in whom the etiological study was not performed were considered possible NB, since the cause of urinary symptoms could not be confirmed.

For the survival analysis, the time from motor symptoms onset until death or tracheostomy was considered the endpoint.

Statistical analysis

Data were summarized by mean, standard deviation, median and first and third quartiles in the case of continuous variables and by relative and absolute frequencies in the case of categorical variables. Association between the different urinary symptoms scales (OABV8, ICIQ-SF and IPSS) on the one hand, and phenotype and ALSFRS-R score on the other, was assessed using ordinal logistic regression models.

Age, gender and time from onset of symptoms were also included in the models as covariates. Cognitive or behavioural impairment were not included since 12 values were lacking, which significantly affected the model. Consequently, we performed an ordinal regression using the presence of cognitive or behavioural impairment as predictor variables and the urinary symptoms scale scores as response variables. Association of the clinical variables with presence of relevant urinary symptoms and NB was assessed using logistic regression models. We performed a multivariable survival analysis to study the effect of urinary symptoms in risk of death of the study population. Survival was calculated since motor symptoms onset. We included as covariates other variables (age, bulbar onset and phenotype) that affect survival in ALS patients (24). A log-rank test was used to assess differences in survival between csLUTS+ and csLUTS- patients in the subgroup of them with less than 24 months of disease evolution at the study onset. P values < 0.05 were considered statistically significant. All statistical analyses and graphs were performed using R software (version 3.2.0).

Results

From May to November 2014, 55 out of 79 patients (70%) visiting the ALS Unit and meeting the inclusion criteria, accepted to participate and were included in this study.

Patient characteristics and urinary symptoms

Table 1 summarizes demographical and clinical characteristics and questionnaires scores in each phenotype. Three patients (2 cALS and 1 PMA) reported family history (5.45%). A *C9ORF72* expansion was found in both cALS patients but no mutation was found in the PMA patient. No *C9ORF72* expansion was found among sporadic patients. Scores on urinary symptoms' questionnaires were similar among phenotypes except for OAB-V8, which was higher in PMA patients. Altogether, twenty-four patients (43.6%), including 2 fMND patients (one *C9ORF72* carrier), reported csLUTS. Most of them reported mixed (storage and voiding) symptoms and more than half of them met criteria of urgency urinary incontinence (ICIQ-SF>0), again mainly with mixed symptoms.

Clinical characteristics of neurogenic bladder (NB) patients

UDS was performed in 10 out of 24 patients reporting csLUTS (5 cALS, 3 PMA and 2 PLS), finding NB in nine (most of them with DSD). All NB+ patients were male and sporadic cases. Two main clinical patterns could be distinguished (Supp Table 1): patients reporting symptoms in the first two years of the disease (case 1 to 5) with urodynamic features of severe neurogenic bladder (16), other extra-motor symptoms (small-fiber neuropathy, dysautonomia or FTD) and a poor survival; and those reporting symptoms later on (case 6 to 9), with milder urodynamic findings and a relatively benign disease course. Fourteen patients reported csLUTS, but UDS were not performed and NB could not be demonstrated (possible NB). A summary of clinical characteristics of NB+, possible NB and NB- patients can be found in Table 2.

Relationship between urinary symptoms and disease characteristics

We first aimed to ascertain if urinary symptoms were a result of the disease phenotype or progression. For that end, we studied with multivariable analysis the relationship between urinary symptoms (ICIQ-SF, OAB-V8 and IPSS scores and the presence or absence of csLUTS) and clinical variables (phenotype, ALSFRS-R score and time from symptoms onset) adjusting for age and sex. Age seemed to marginally influence OAB-V8 score (OR = 1.06, p = 0.04), whereas female sex showed a tendency to be a protective factor for csLUTS (OR = 0.39, p = 0.06). None of the clinical variables showed a relevant effect on questionnaires' scores neither on the presence of csLUTS (Table 3). Afterwards, we studied the association of dysexecutive or behavioural impairment on urinary symptoms and found no association with OABV8 score (OR = 1.06 [0.96, 1.18], p = 0.26), ICIQ-SF score (OR = 1.18 [0.98, 1.49], p = 0.11), IPSS score (OR = 1.04 [0.94, 1.14], p = 0.46) or csLUTS (OR = 0.96 [0.28, 3.25], p = 0.94). Moreover, rates of cognitive or behavioural impairment between csLUTS and non-csLUTS patients were similar (38.1% vs 39.1%).

Relationship between urinary symptoms and disease prognosis

Fig. 1 represents patients' survival, according to csLUTS and NB status. Survival of csLUTS was similar to non-csLUTS patients (70.6 vs 62.9 months, p = 0.69). More NB+ patients met the endpoint (death or tracheostomy) at last follow-up, and tracheostomy free survival was shorter in these patients vs NB- (70.6 vs 28.2 months respectively), although it was not statistically significant (p = 0.19). As stated above, we

noticed that patients showing NB within two years from symptoms onset deteriorated rapidly and met the endpoint prematurely. Conversely, we also observed that some other patients reporting csLUTS (mainly those with PMA and PLS phenotypes) were already long survivors (> 4 years) at the time of recruitment. We therefore hypothesized that LUTS appear because of disease spread, usually after some years of disease course, but whenever they appear early (< 2 years) on the disease course, they could predict poor survival. To confirm this hypothesis, we performed a multivariable survival analysis adjusting for age, phenotype (cALS, PMA, PLS) and region of onset (bulbar vs spinal), and introduced an interaction between urinary symptoms and the disease duration at the time of recruitment. Table 4 shows the result of the regression model fitted to study the effect of urinary symptoms in the risk of death.As expected, a longer duration of motor symptoms at the study recruitment associated to longer survival ($p = 0.007$), since many of those patients were already long survivors at the time of recruitment. Moreover, age and urinary symptoms increased the risk of death, after adjusting by disease duration, phenotype and region of symptoms onset, although statistical significance was not met ($p = 0.06$ and $p = 0.08$ respectively).

Discussion

Although severity of motor impairment had led to underestimate other symptoms, MND is now recognized as a heterogeneous multisystem disorder, in which non-motor symptoms (including LUTS) are frequent (2,3,14–16). In this study, we found evidences that early and severe neurogenic bladder in MND patients, usually associates with other non-motor symptoms and could be a marker of poor prognosis.

Factors associated to csLUTS

Medication or reduced mobility can contribute to LUTS in ALS, resulting in functional incontinence. However, we had previously shown that, in most patients, LUTS were due to neurogenic bladder (16). In this extension study, with the same cohort of patients, we found no association between LUTS and disability (measured with ALSFRS-R) or disease duration, confirming that reduced mobility is not a major explanation for LUTS as previously suggested (14). We had previously reported that clinical and demographical characteristics of csLUTS and non-csLUTS patients were similar, although males seemed to be overrepresented in the csLUTS group (16). This trend is

now still evident after the multivariate analysis, where male sex was the only studied variable that appeared to predispose to csLUTS. Furthermore, we found no evidence of association between dysexecutive or behavioural impairment and urinary symptoms.

A previous report found a weak association of age and predominant UMN impairment with UI (14). In our study, age was associated to OAB-V8 scores but not to other scores or overall to csLUTS, suggesting that age can influence some LUTS but not others. Moreover, although we did not specifically study the association of UMN signs and LUTS, we found no relationship between phenotype and any of the studied urinary scores, and the frequency of csLUTS and NB was similar across phenotypes.

Pathogenesis of csLUTS

Lesions in different nervous system structures can prompt LUTS. A suprapontine damage affecting frontal lobe and/or striatum (after a stroke, in frontotemporal dementia or Parkinson's disease) frequently causes storage symptoms secondary to detrusor overactivity, although DSD or uninhibited sphincter relaxation have also been reported (9,10). Infrapontine suprasacral lesions (such as those found in multiple sclerosis or spinal cord injury) usually result in both storage and voiding symptoms secondary to DSD (9,10). Finally, sacral or infrasacral lesions (e.g., the cauda equine syndrome) cause predominantly voiding symptoms as a consequence of hypocontractile detrusor with or without an underactive sphincter (9,10). In our study, most csLUTS patients reported both storage and voiding symptoms, the post-void residual was raised and DSD was the most frequent finding in the UDS, suggesting an infrapontine suprasacral lesion as the cause of LUTS in MND patients. The nature and prevalence of csLUTS and the results of UDS were similar across phenotypes (cALS, PMA and PLS), which suggests a common pathogenic mechanism. This is not surprising since other extra-motor features such as cognitive impairment have also been reported to be similar across phenotypes (25,26). However, particularities in some individuals or the concomitant involvement of several structures as a cause of LUTS cannot be ruled out. For example, ALS patients carrying the L106V *SOD1* mutation and experiencing LUTS, showed widespread (suprapontine, infrapontine and sacral) neuronal loss and gliosis (27). Neuroimaging and pathologic studies are warranted to find out anatomic correlates of LUTS in ALS.

Clinical characteristics of NB+ patients

Despite all NB+ patients were male, benign prostatic enlargement was ruled out by PSA and ultrasound measurement, as a possible explanation to csLUTS. Possible NB patients were slightly more disabled and had longer disease duration than NB+ patients, suggesting that patients with higher disability were more prone to reject UDS despite the presence of csLUTS. As found in csLUTS patients, the UMN score and rates of dysexecutive and behavioural impairment were not different in NB+ patients. Moreover, multivariable analysis of factors leading to NB was not performed because of the small sample in each group. Among NB+ patients we noticed two clinical patterns. First, patients reporting symptoms in the first two years of the disease who usually showed severe neurogenic bladder (16), other extra-motor features and a poor survival. Second, those reporting symptoms later on the disease course, with milder urodynamic findings and a relatively benign disease course.

Survival analysis

In ALS, the extent to which different neuronal networks are affected varies significantly between patients, which affects the clinical picture and prognosis (28). For example, cognitive impairment seems to appear early in the disease course and worsen only in subjects with previous cognitive impairment, but not in those cognitive normal patients (29). Furthermore, ocular movements' abnormalities are a subclinical common feature that worsens in some patients during disease progression but can remain intact in others (30). Interestingly, patients who develop cognitive impairment early in the disease course, show faster rates of motor functional decline and shorter survival (29). Similarly, subjects requiring early tracheostomy, were those who most commonly lost their ability to communicate by any means (30). These data suggest that patients showing early spread to non-pyramidal structures have also faster deterioration of motor functions. Consistent with this hypothesis, we observed a trend to shorter survival in NB+ and csLUTS patients reporting symptoms in the first two years of disease. This trend was confirmed in the multivariable survival analysis, when accounting for the interaction between disability and disease duration, as well as for other previously reported risk factors such as bulbar onset, age or phenotype (24). However, these results must be interpreted with caution, since statistical significance

was not met. Remarkably, most NB+ patients reporting csLUTS in the first two years of disease, showed also other extra-motor features, suggesting a broader disease spread in this patients.

Genetic factors account at least partially for different phenotypes and prognosis (28). Interestingly, LUTS have been recently shown to be frequent in ALS patients carrying the L106V *SOD1* mutation, and they occurred simultaneously with the need of an artificial ventilator (27), suggesting that some genetic factors could influence the appearance of LUTS and that this event could be, in a certain way, related to prognosis. In our study, csLUTS or NB could not be explained by fMND. Moreover, although less than 5% of sporadic patients carry a MND-causal mutation (31), we analysed C9ORF72 in both sporadic and familial MND without observing any relationship between genotype and the phenotypes analysed here. Therefore, Mendelian gene mutations are unlikely to explain LUTS in our population. However, this does not exclude that some mutations can predispose to LUTS and our results should be taken with caution, because the relatively small studied sample. Larger studies with a more comprehensive genetic testing are warranted to establish the role of genetics in non-motor symptoms in MND.

Limitations and strengths

This study has several limitations. First, recruitment was cross sectional and the date of onset of LUTS was not collected, which hinders the interpretation of the survival analysis. Prospective longitudinal studies are warranted to establish the timing of LUTS onset in MND patients and to confirm a possible association between early csLUTS and survival. Second, the sample of studied patients was relatively small. However, this is the largest and most thorough study of urinary symptoms in MND up to now (14,15). Furthermore, this is the first study describing characteristics of MND patients with confirmed neurogenic bladder. Third, cognitive examination could not be performed in all patients and was focused on dysexecutive impairment . However, we avoided the influence of motor symptoms on cognitive measurements and we used Spanish normative data. Cognitive examination in MND is a developing field but the dysexecutive syndrome plays a pivotal role in the definition of cognitive impairment

(22). At the time in which the study was performed, there were no MND-specific questionnaires validated in Spanish population, so we selected those executive tests more widely used in MND population (22).

In conclusion, urinary symptoms are frequent in ALS patients, especially in males, but regardless of genotype, phenotype, site of onset, cognitive impairment or disability. They can occur as an early or late feature and they could be the result of infrapontine impairment. When occurring early, they usually lead to severe NB and are accompanied by other non-motor symptoms. In those cases, they could be a sign of rapid spread of the disease and consequently they could predict a poor prognosis. Further prospective longitudinal studies including genetic and neuroimaging analysis are warranted to confirm this hypothesis.

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Conflicts of interest: None.

Contributors

JFVC designed the study, participated in clinical data acquisition and interpretation, and wrote and edited the manuscript. SA co-designed the study, participated in clinical data acquisition and interpretation, and critically revised the manuscript. DH participated in data analysis and interpretation, and critically revised the manuscript. EMC and EB participated in clinical data acquisition and critically revised the manuscript. TS participated in clinical data acquisition, supervised the study and critically revised the manuscript. FC and JPT performed the genetic analysis and critically revised the manuscript. JFVC had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors have approved the submitted version of the paper.

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	cALS	PMA	PLS
Total n (%) from 55 patients	37 (67%)	10 (18%)	8 (15%)
Age (years) *Mean (SD)* *Median (IQR)*	 60.89 (10.82) 60.15 (53.26, 68.38)	 69.4 (8.47) 70.66 (61.76, 76.78)	60.8 (11.58) 63.66 (54.62, 67.98)
Sex (male) n (%)	21 (56.76%)	8 (80%)	4 (50%)
Time from motor symptoms onset (months) *Mean (SD)* *Median (IQR)*	 29.58 (20.54) 24.2 (13.03, 44.13)	 60.82 (85.46) 36.86 (13.45, 52.13)	105.78 (88.04) 66.45 (39.53, 157.69)
Symptoms onset Bulbar n (%) Spinal n (%)	 8 (21.6%) 29 (78.4%)	 0 (0%) 10 (100%)	 1 (12.5%) 7 (87.5%)
ALSFRS-R *Mean (SD)* *Median (IQR)*	 32.05 (8.49) 34 (26, 38)	 30.8 (9.4) 33 (27.5, 34.75)	 27.12 (10.47) 31.5 (16, 35.5)
MRC *Mean (SD)* *Median (IQR)*	 94.92 (25.87) 102.5 (70.25, 116.5)	 91.2 (27.9) 100.5 (68.5, 115.75)	94.38 (51.06) 127.5 (65, 128.5)
UMN score *Mean (SD)* *Median (IQR)*	 6.86 (4.61) 6 (3, 10)	 0.1 (0.32) 0 (0, 0)	12.62 (2.5) 13.5 (11.5, 14.25)
Progression rate *Mean (SD)* *Median (IQR)*	 0.87 (0.84) 0.61 (0.27, 1.08)	 0.6 (0.43) 0.42 (0.36, 0.82)	0.26 (0.11) 0.26 (0.15, 0.35)
Cognitive and or behavioural impairment, n= 44 Yes n (%)	 12 (40%)	 2 (25%)	 1 (16.7%)
Frontotemporal dementia, n=55 Yes n (%)	 2 (5.4%)	 0 (0%)	 0 (0%)
Endpoint (death or tracheostomy) n (%)	21 (56.8%)	4 (40%)	1 (12.5%)
Survival (months) Median (IQR)	 41.77 (28.2, 62.4)	 60.48 (32.34, 69.22)	92.18 (65.5, 184.41)
ICIQ-SF, n=55 *Mean (SD)* *Median (IQR)*	 2.08 (4.67) 0 (0, 0)	 1.9 (3.28) 0 (0, 3)	 1.12 (2.23) 0 (0, 0.75)
OAB-V8, n= 54 *Mean (SD)* *Median (IQR)*	 5.81 (7.57) 4 (0, 9)	 9 (8.15) 8.5 (1, 16.25)	 5.86 (6.2) 3 (1, 10)

IPSS total score, n=50 *Mean (SD)* *Median (IQR)*	 5.53 (6.68) 2.5 (1, 8.75)	 6.1 (7.43) 3.5 (0.5, 9.5)	 7 (7.53) 3 (2, 9.5)
IPSS storage, n=50 *Mean (SD)* *Median (IQR)*	 3.41 (3.4) 2 (1, 4)	 4.57 (4.47) 3 (2, 5)	 2.29 (2.69) 1 (0.5, 3.5)
IPSS voiding, n=50 *Mean (SD)* *Median (IQR)*	 2.8 (4.37) 1 (0, 3.75)	 4.14 (4.06) 2 (1, 7.5)	 4.71 (5.71) 3 (0.5, 7)
QoL- IPSS *Mean (SD)* *Median (IQR)*	 0.93 (1.25) 0 (0, 1)	 1.8 (1.81) 1 (0.25, 3)	 1.33 (1.37) 1 (0.25, 2.5)
csLUTS n (%)	14 (37.83%)	6 (60%)	4 (50%)
csLUTS patients with IPSS>7, n (%) - Storage - Voiding - Mixed	 10 (71.43%) 1 (10%) 2 (20%) 7 (70%)	 4 (66.67%) 4 (100%)	 3 (75%) 1 (33.33%) 2 (66.67%)
Urinary incontinence (ICIQ-SF>0) n (%)	8 (21.62%)	3 (30%)	2 (25%)
Urodynamic study n (%)	5 (13.5%)	3 (30%)	2 (25%)
Result of urodynamic study	4 DSD 1 Normal	2 DSD 1 Obstructive	1 DSD 1 Obstructive + Hypocontractile
Neurogenic bladder, n (%) No Possible Definite	 24 (64.9%) 9 (24.3%) 4 (10.8%)	 4 (40%) 3 (30%) 3 (30%)	 4 (50%) 2 (25%) 2 (25%)

Table 1. Demographical and clinical characteristics of patients included in the study as per phenotype. cALS: classical ALS; csLUTS: clinically significant lower urinary tract symptoms; DSD: detrusor-sphincter dyssynergia; MRC: Medical Research Council; PLS: primary lateral sclerosis; PMA: progressive muscular atrophy; UMN: upper motor neuron.

	NB – (n=32)	NB Possible (n=14)	NB + (n=9)
Age (years)			
Media (SD)	61.49 (11.66)	65.24 (9.31)	61.38 (10.62)
Median (IQR)	60.67 (55.13, 68.71)	65.95 (58.87, 71.19)	63.47 (61.2, 66.46)
Sex (male)			
n (%)	15 (46.9%)	9 (64.3%)	9 (100%)
Phenotype			
PMA	4 (40%)	3 (30%)	3 (30%)
ALS	24 (64.9%)	9 (24.3%)	4 (10.8%)
PLS	4 (50%)	2 (25%)	2 (25%)
Diabetes mellitus, n (%)			
NO	25 (78.1%)	13 (92.9%)	8 (88.9%)
YES	7 (21.9%)	1 (7.1%)	1 (11.1%)
Disease duration (months)			
Media (SD)	41.99 (44.46)	66.85 (88.81)	29.96 (20.06)
Median (IQR)	28.78 (13.82, 51.78)	37.1 (17.63, 64.1)	27.8 (12.23, 51.13)
ALSFRS.R			
Media (SD)	31.44 (8.21)	29.29 (10.82)	32.78 (8.9)
Median (IQR)	33.5 (26, 37)	32 (18.75, 37.25)	35 (28, 39)
MRC			
Media (SD)	91.77 (29.25)	92.71 (35.86)	104.56 (25.57)
Median (IQR)	96 (69.5, 116)	109 (55.75, 121.75)	118 (76, 125)
UMN.Score			
Media (SD)	6.38 (5.07)	7.5 (6.07)	5.22 (5.24)
Median (IQR)	5.5 (2.75, 10.25)	9 (1.25, 12.75)	6 (0, 8)
Region of onset, n (%)			
Bulbar	6 (18.8%)	2 (14.3%)	1 (11.1%)
Spinal	26 (81.2%)	12 (85.7%)	8 (88.9%)
Axial	0 (0%)	1 (7.1%)	1 (11.1%)
Generalized	4 (12.5%)	0 (0%)	1 (11.1%)
Lower limbs	13 (40.6%)	8 (57.1%)	5 (55.6%)
Upper limbs	9 (28.1%)	3 (21.4%)	1 (11.1%)
Side of onset, n (%)			
Dominant	17 (53.1%)	6 (42.9%)	3 (33.3%)
Non-dominant	13 (40.6%)	7 (50%)	4 (44.4%)
Symmetric	2 (6.2%)	1 (7.1%)	2 (22.2%)
Cognitive or behavioural impairment, n=44			
Mild impairment, n (%)	9 (37.5%)	2 (15.4%)	3 (42.8%)
FTD, n (%)	1 (4.2%)	0 (0%)	1 (14.3%)
NO, n (%)	14 (58.3%)	11 (84.6%)	3 (42.8%)

Progression rate Media (SD) Median (IQR)	 0.66 (0.6) 0.5 (0.27, 0.82)	 0.75 (0.8) 0.37 (0.14, 1.28)	 0.93 (1.08) 0.53 (0.28, 1.15)
Death or tracheostomy, n (%) NO YES	 18 (56.2%) 14 (43.8%)	 8 (57.1%) 6 (42.9%)	 3 (33.3%) 6 (66.7%)

Table 2. Clinical characteristics of patients according to the presence or absence of neurogenic bladder. ALS: amyotrophic lateral sclerosis; FTD: Frontotemporal dementia; MRC: medical research council; PLS: primary lateral sclerosis; PMA: progressive muscular atrophy; UMN: upper motor neuron.

	ICIQ		OAB-V8		IPSS		csLUTS	
	OR [CI]	p	OR [CI]	p	OR [CI]	p	OR [CI]	p
PMA	0.97 [0.16, 4.77]	0.97	0.74 [0.16, 3.27]	0.69	0.69 [0.15, 2.99]	0.62	1.96 [0.40, 10.67]	0.41
PLS	1.28 [0.14, 9.21]	0.81	0.95 [0.16, 5.23]	0.96	1.44 [0.31, 6.81]	0.64	2.68 [0.42, 18.60]	0.29
Age	1.03 [0.96, 1.11]	0.38	1.06 [1.00, 1.12]	0.04*	1.03 [0.98, 1.09]	0.20	1.03 [0.97, 1.10]	0.35
Sex (Woman)	0.46 [0.10, 1.81]	0.28	0.47 [0.15, 1.35]	0.17	0.38 [0.13, 1.14]	0.09	0.39 [0.08, 1.03]	0.06
ALSFRS-R	1.01 [0.93, 1,10]	0.80	1.00 [0.93, 1.06]	0.89	0.99 [0.92,1.05]	0.69	0.99 [0.92, 1.07]	0.83
Time from symptoms onset	0.92 [0.38, 2.17]	0.84	1.12 [0.58, 2.18]	0.74	0.81 [0.41, 1.59]	0.53	0.72 [0.32, 1.57]	0.42

Table 3. Relationship between urinary symptoms (ICIQ-SF, OAB-V8 and IPSS scores and csLUTS) and clinical variables adjusting for age and sex. csLUTS: clinically significant lower urinary tract symptoms; PLS: primary lateral sclerosis; PMA: progressive muscular atrophy; * statistically significant.

	Exp (coef)	Lower 0.95	Upper 0.95	p
csLUTS	21.5	0.66	906.86	0.08
Disease duration	0.23	0.07	0.67	0.007*
Age	1.05	0.977	1.1	0.06
Region of onset (spinal)	0.98	0.36	3.21	0.97
Phenotype (PMA)	0.61	0.18	1.75	0.38
Phenotype (PLS)	0.26	0.02	1.29	0.11
csLUTS : Disease duration	0.41	0.13	1.19	0.10

Table 4. Multivariable survival analysis. csLUTS: clinically significant lower urinary tract symptoms; PLS: primary lateral sclerosis; PMA: progressive muscular atrophy; * statistically significant.

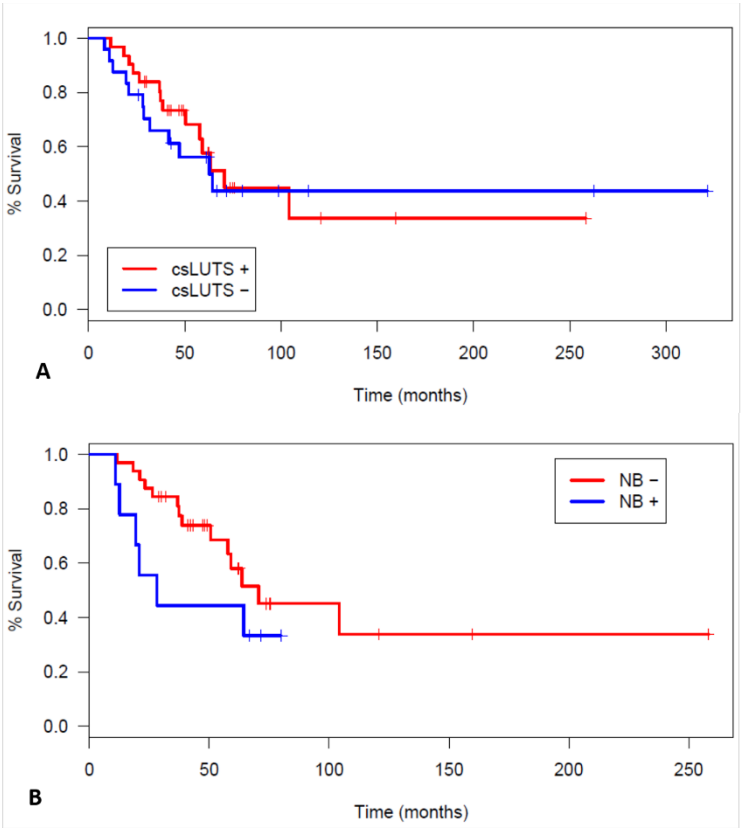

Fig. 1 Cumulative survival of ALS patients with or without csLUTS (A) and with or without NB (B). ALS: amyotrophic lateral sclerosis; csLUTS: clinically significant lower urinary tract symptoms; NB: neurogenic bladder.

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Genetic and constitutional factors are major contributors to *substantia nigra* hyperechogenicity

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Hyperechogenicity of *substantia nigra* (SNh) is a frequent finding in amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and other movement disorders (MD) patients, but its meaning is unclear. To ascertain the contribution of different factors to SNh area, we measured it in 108 ALS, 102 PD, 91 other MD patients and 91 healthy controls. Demographical data were collected in all patients and controls. In ALS patients, we also recorded clinical variables, performed genetic analysis and measured baseline levels of ferritin. After family history and genetic testing, ALS patients were classified as familial (15) or sporadic (93). ALS, PD and other MD patients had a larger SNh area than controls. Left SNh and male gender, but not age, associated with larger SNh area in both patients and controls. Familial ALS patients showed larger SNh area than sporadic ones and familial ALS was the only clinical variable in the multivariate analysis to be associated with larger SNh area in ALS patients. Our results suggest that SNh associates with genetic and constitutional factors (male gender, handedness), some of which predispose to certain neurodegenerative diseases. This evidence supports the idea of SNh as an inborn marker of unspecific neuronal vulnerability.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease clinically characterised by progressive weakness, and by signs of upper (UMN) and lower motor neuron (LMN) impairment. Three different phenotypes can be distinguished according to the degree of clinical impairment of UMN and LMN[1]: classical ALS (cALS), primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA). Pathologically, ALS is characterised by TDP-43 aggregates found in degenerating UMN and LMN[1]. However, TDP-43 deposits and a variable degree of neuronal loss can be found far beyond motor neurons in many ALS patients; e.g., *substantia nigra* (SN) in about 50% of them[2,3]. The extension of these deposits probably accounts for the presence or absence of extra-motor features, such as cognitive or behavioural impairment in ALS patients[1].

By means of transcranial sonography (TCS), a larger area of increased echogenicity (hyperechogenicity) in SN has been found in patients with different neurodegenerative diseases (especially in Parkinson's disease, PD) compared to controls[4]. This SN hyperechogenicity (SNh) is thought to reflect increased iron deposits[5]. Although its exact meaning remains unknown, it has been proposed to be a marker of SN degeneration or vulnerability[5]. Recently, SNh has been reported in 50–70% of cALS patients[6–9], but whether in ALS this finding also reflects vulnerability of the nigrostriatal system or the pathologic spread of the disease to the SN is not known. Moreover, SNh has not been studied in other phenotypes (PMA or PLS) or in familial ALS.

The aim of this study was to evaluate the SNh in ALS patients (including PMA and PLS) compared to movement disorders (MD) patients and healthy controls. We also aimed to evaluate the contribution of demographical, clinical, biochemical and genetic factors to SNh in patients and controls.

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Methods

Subjects and definitions. For this cross-sectional study, patients diagnosed with classical ALS (cALS), PMA or PLS who came to our ALS Unit between February 2014 and September 2016 and gave written informed consent, were recruited. Patients are routinely evaluated by the same neurologist (JFVC), and demographical and clinical data are prospectively recorded in a database. The cALS patients met the El Escorial revised criteria of possible, probable or definitive ALS[10]. PMA was defined as a progressive isolated impairment of LMN in at least two regions[11], and PLS as a progressive isolated impairment of UMN in at least one region other than the lumbar region[12].

For comparison purposes, we included a previously reported cohort of subjects without neurodegenerative diseases[13]. We also included patients with MD in whom an increased SNh area had been previously reported[14–16]: PD, atypical parkinsonism, vascular parkinsonism and essential tremor. The data of a subset of these patients have also been previously published[17].

Family history and genetic analysis. ALS patients were systematically asked for family history of ALS, dementia or parkinsonism of any kind in first- or second-degree relatives. When the history of dementia was compatible with frontotemporal dementia (FTD), medical records of this relative were reviewed (whenever available). Patients were categorised as familial ALS (fALS) whenever they met the criteria for possible, probable or definite ALS[18], or as sporadic ALS (sALS) when not. Both sALS and fALS patients were screened for *C9ORF72* with repeat primed PCR, as previously reported[19]. In those fALS patients not carrying a *C9ORF72* expansion, *SOD1*, *TARDBP* and *FUS* genes were subsequently analysed by Sanger sequencing. After the genetic analysis, fALS grades were appropriately reassessed[18].

Clinical and analytical variables. Age, gender, time of symptom onset, region of onset (bulbar and spinal), side of onset (or side predominance in bulbar onset cases) and body mass index before disease onset (premorbid BMI) were recorded for all ALS patients.

ALS patients were examined at the time of recruitment, and the following items were recorded: parkinsonism signs, disability (ALSFRS-R)[20], and degree of UMN impairment (UMN score) for a maximum of 16[21].

Executive and behavioural examination were performed in a subset of 100 patients. Executive function was assessed with digit span; verbal fluency index[22] and stroop test in patients without relevant bulbar symptoms; and trail A and B in patients without symptoms in the dominant hand. Patients were studied with at least two different executive tests, depending on their disability. Mild executive impairment was diagnosed according to current criteria[22]. Relatives were systematically asked for behavioral symptoms following Rascovsky criteria and mild behavioural impairment and FTD were diagnosed according to current criteria[22]. Moreover, 60 patients were assessed with the Spanish validated version of the Frontal Systems Behaviour Scale (FrSBe)[23], fulfilled by the caregiver. The FrSBe has three subscales: apathy, disinhibition, and executive dysfunction. For the present study, we have considered the Z-scores of the post-illness forms.

The basal (within two months from recruitment) levels of ferritin were determined in 98 patients by immunoturbidimetry on a Beckman Coulter AU 5400 according to manufacturer's instructions.

TCS examination. A TCS examination was performed by an expert neurologist sonographer (JIT), who was blind to the clinical data, with the same ultrasound system (Toshiba Aplio XG, Tokyo, Japan 2008) equipped with a 2.5 MHz phased-array transducer to obtain B-mode images through a temporal acoustic bone window. Right and left SNh were obtained and measured by this examiner, as previously published[13].

Statistical analysis. Differences in the right and left SNh areas in patients and controls (age- and gender-adjusted) were assessed with a linear mixed regression model. Differences in the SNh area in fALS and sALS were assessed by the Wilcoxon test, and the relationship between SNh and ferritin, verbal fluency index and FrSBe Z-scores was assessed by Pearson's correlation. To assess the effect of side of disease onset on the size of contralateral SNh, linear model regression was performed, which included an interaction between these covariates. An association of the demographical and clinical variables of the ALS patients with SNh area on both sides was assessed by a mixed linear regression model. In this model was assumed that SNh area depended on fixed effects (covariates) and random effects due to inter-individual variability. Firstly, a group of covariates (age, gender, family history, fALS, premorbid BMI, disease duration, phenotype, ALSFRS-R, executive or behavioural impairment, parkinsonism and SNh side) were pre-selected based on previous literature and the study goal. Finally, the combination of covariates that best fitted the model was selected according to the Akaike Information Criteria (AIC). P values of <0.05 were considered statistically significant. All the statistical analyses and graphs were performed using version 3.2.2 of the R software.

Ethical approval. The study was approved by the Ethics Committee for Biomedical Research of the La Fe Hospital (Valencia, Spain) and has been conducted according to the principles of the Declaration of Helsinki. All the participants gave written informed consent.

Data availability. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Results

Population characteristics. One hundred and eight ALS patients (74.1% cALS, 15.7% PMA and 10.2% PLS) and 193 MD patients (102 PD and 91 with other MD) were recruited for this study. Ninety-one healthy controls, in whom at least one SNh was measureable, were also included. Overall ALS patients were moderately disabled and the time since symptoms onset ranged between 17 months in ALS to 49 months in PLS. The

demographical and ultrasound characteristics of both patients and controls, and the clinical and genetic characteristics of ALS patients as per phenotype, are summarised in Table 1.

Family history and genetic analysis. Unspecified dementia was the most frequently reported family history in ALS patients, followed by ALS or FTD (Table 1). Thirteen (12%) ALS patients of 11 different families (three members of one family were included in the study) were classified as fALS as per history[18]. A pathologic expansion in *C9ORF72* was found in seven of the 11 (64%) index fALS cases. A *SOD1* mutation was found in three index fALS cases (p.E22G and p.N139H) and in two affected relatives of the index case carrying the p.N139H mutation. No mutations were identified in one fALS case. *C9ORF72* was found in two of the 95 sporadic cases (2%). Consequently, 15 ALS patients (13.9%) were, after genetic analysis, classified as fALS[18].

SNh area in patients and controls. It was possible to measure SNh area through the temporal acoustic bone window in at least one side in 97 (89.8%) ALS patients (including 12 of 15 fALS patients, 80%), 93 (91.2%) PD patients and 73 (80.2%) patients with other MD.

cALS, PMA and PLS patients and the MD patients showed a larger SNh area than the healthy controls. The largest mean and median SNh areas were found in PD patients and the smallest ones in PLS patients (Table 1). Left SNh area was greater than the right one in all cohorts, except in PMA patients (Table 1, Fig. 1). Based on our previous report[13], hyperechogenicity of SN (SNh+) was considered whenever one SNh area was larger than 0.22 cm^2, whereas SNh− was considered whenever both areas were smaller than 0.22 cm^2. Patients with only one visible SNh area, which was $<0.22\ cm^2$ were excluded because their classification was uncertain. Accordingly, SNh+ was found in 56.7% of ALS patients compared to 8.8% of healthy controls (Table 1).

Contribution of demographical variables to SNh area in patients and controls. In the multivariate analysis, differences in SNh area between ALS patients and the MD controls on one side and healthy controls on the other were statistically significant after adjusting for age, gender and side of SNh (Table 2). Left SNh and male gender, but not age, were associated with a larger SNh area in the multivariate analysis (Table 2). This effect of male gender and left side was apparent in each subgroup of patients and controls (Table 1, Fig. 1).

Associaton of demographical, clinical and biochemical variables with SNh area in the ALS patients. Age, gender, mutations, familial history and disease duration showed differences in the SNh+ compared to the SNh− ALS patients (Table 3). Conversely, no differences in disease characteristics were apparent, except for executive or behavioural impairment and parkinsonism, which were slightly more frequent in the SNh+ patients.

In the univariate analysis, fALS patients presented a larger SNh area than the sporadic patients (0.20 *vs*. 0.28, $p = 0.01$). This effect appeared to be independent of the causing mutation (Fig. 2). However, no correlation was found between the SNh and basal levels of ferritin in the ALS patients ($R = -0.0005$, $p = 0.99$) nor between SNh and verbal fluency index or any of the FRSBe subscales (Supplementary Table 1).

We found no effect of disease lateralisation on the size of contralateral SNh in ALS patients (estimate $= -0.004$, $p = 0.91$), and the left SNh area appeared to be larger independently of side of disease onset (Supplementary Table 2 and Supplementary Figure 1).

In the multivariate analysis, we first analysed all the pre-selected covariates. Subsequently, we repeated the analysis after removing those covariates that were not found to be significant in the first model, and for which there were no previous evidences of association with SNh (parkinsonism, ALSFRS-R, cognitive or behavioural impairment, disease duration and premorbid BMI). The model showed notable improvement (AIC −161 *vs*. −233). In this final model, fALS was the only variable that was significantly associated with a larger SNh area in ALS patients, but a trend to negative association for PLS and to positive association for left SNh side (Table 4) were observed.

Discussion

Firstly thought to be a marker of neuronal degeneration in the SN, more recent studies suggest the SNh role as a marker of SN vulnerability[5,24]. Our study strongly reinforces this hypothesis and adds further evidences that genetic and constitutional factors are key to predispose to SNh in different neurodegenerative diseases and controls.

We studied a cross-sectional cohort of patients in an ALS Unit of a tertiary centre in Spain. This explains why some phenotypes (PMA and PLS) and fALS cases can be slightly overrepresented compared to prospective population-based studies. Rates of fALS and causing mutations in our cohort were similar to those previously described in European population[25].

In our study, SNh area was found larger in all the studied diseases compared to healthy controls after adjusting for age and gender. PD patients presented the largest area, whereas patients with other MD and PLS patients had the smallest SNh area. Although an increased SNh area was first described in PD patients, it has also been found in other MD[14–16], as well as in neurodegenerative diseases not typically characterised by SN degeneration or vulnerability, such as ALS or Friedreich's ataxia[7,8,26]. Our data are consistent with those reports showing that, although PD patients are those with larger SNh, SN+ is also a frequent finding in different ALS phenotypes and other MD.

Male gender strongly associated with a larger SNh area in the entire population, and this association was apparent for each studied cohort. However, they lost significance when analysed only in ALS patients and together with other clinical variables, which also influenced SNh area. This is probably the result of having a lower statistical power due to the reduced sample size, since a trend of this effect was still evident. The effect of male gender on SNh area has been previously reported in PD and controls[5,24,27], which further supports our results. Interestingly, male gender is more overrepresented in PD, cALS and PMA, but not in PLS[24,28]. This suggests that

	cALS (n = 80)	PMA (n = 17)	PLS (n = 11)	PD (n = 102)	OMD (n = 91)	Controls (n = 91)
Age (years)						
Mean (*SD*)	61.75 (12.26)	63.98 (13.2)	64.71 (11.68)	77.1 (88.15)	70.98 (9.58)	66.92 (12.7)
Median (*IQR*)	61.37 (54.93, 71.13)	65 (59.76, 72.07)	68.48 (58.95, 73.07)	70 (62.25, 74)	72 (65, 79)	69 (59, 76)
Gender (male)						
n (%)	45 (56.2%)	15 (88.2%)	8 (72.7%)	60 (58.8%)	51 (56%)	67 (73.6%)
Premorbid BMI, n = 102						
Mean (*SD*)	27.61 (4.53)	27.78 (4.49)	27.13 (3.64)			
Median (*IQR*)	27.24 (24.68, 29.76)	26.53 (24.67, 30.58)	28.07 (24.75, 29.52)			
Family history						
Dementia n (%)	10 (12.5%)	3 (17.6%)	0 (0%)			
PD n (%)	3 (3.8%)	0 (0%)	0 (0%)			
Dementia and PD n (%)	3 (3.8%)	0 (0%)	0 (0%)			
ALS or FTD n (%)	10 (12.5%)	3 (17.6%)	1 (9.09%)			
Region of onset						
Bulbar n (%)	22 (27.5%)	1 (5.9%)	3 (27.3%)			
Spinal n (%)	58 (72.5%)	16 (94.1%)	8 (72.7%)			
Side of onset or side predominance, n = 106						
Right n (%)	33 (41.2%)	8 (47.1%)	3 (27.3%)			
Left n (%)	36 (45%)	5 (29.4%)	5 (45.5%)			
Symmetric n (%)	10 (12.5%)	3 (17.6%)	3 (27.3%)			
Parkinsonism						
n (%)	4 (5%)	0 (0%)	1 (9.1%)			
Time from onset (months)						
Mean (*SD*)	23.46 (20.05)	42.69 (42.57)	75.38 (66.6)			
Median (*IQR*)	16.75 (8.78, 29.82)	25.03 (14.07, 55.8)	48.83 (37.45, 99.1)			
ALSFRS-R						
Mean (*SD*)	34.91 (7.74)	37.71 (6.72)	32.3 (7.21)			
Median (*IQR*)	35 (31, 41.25)	39 (36, 43)	34.5 (29.25, 36.75)			
UMN score						
Mean (*SD*)	5.23 (5.03)	0 (0)	12.4 (3.03)			
Median (*IQR*)	3 (1, 9)	0 (0, 0)	13.5 (12.25, 14)			
EB impairment (n = 100)						
No	46 (61.3%)	12 (80%)	3 (30%)			
Mild	24 (32%)	3 (20%)	7 (70%)			
FTD	5 (6.7%)	0 (0%)	0 (0%)			
Mutation carriers						
C9ORF72	8 (10%)	0 (0%)	1 (9.09%)			
SOD1	2 (2.5%)	3 (17.6%)	0 (0%)			
Familial ALS	11 (13.7%)	3 (17.6%)	1 (9.09%)			
Definite	2	1	1			
Probable	6	2				
Possible	3					
Right SNh area						
Mean (*SD*)	0.2 (0.13)	0.21 (0.1)	0.14 (0.08)	0.24 (0.13)	0.16 (0.11)	0.07 (0.08)
Median (*IQR*)	0.18 (0.11, 0.26)	0.19 (0.18, 0.26)	0.09 (0.08, 0.18)	0.24 (0.15, 0.3)	0.15 (0.08, 0.24)	0.06 (0, 0.11)
Left SNh area						
Mean (*SD*)	0.23 (0.12)	0.22 (0.12)	0.17 (0.12)	0.29 (0.14)	0.2 (0.14)	0.08 (0.09)
Median (*IQR*)	0.22 (0.14, 0.29)	0.2 (0.12, 0.3)	0.18 (0.06, 0.28)	0.27 (0.2, 0.38)	0.18 (0.09, 0.29)	0.06 (0, 0.12)
Left SNh area predominance						
n (%)	32 (53.3%)	6 (46.2%)	6 (75.5%)	44 (54.2%)	45 (56.2%)	40 (70.2%)
SN+, n (%)	39/71 (54.9%)	9/15 (60%)	4/11 (36.4%)	69/93 (74.2%)	31/73 (42.5%)	8 (8.8%)

Table 1. Demographical, clinical and ultrasound characteristics of patients (as per phenotype) and controls. ALS: amyotrophic lateral sclerosis; BMI: body mass index; cALS: classical ALS; EB: executive or behavioural; FTD: frontotemporal dementia; OMD: other movement disorders (atypical parkinsonism, vascular parkinsonism and essential tremor); PD: Parkinson's disease; PLS: primary lateral sclerosis; PMA: progressive muscular atrophy; SNh: hyperechogenicity of substantia nigra; SN+: hyperechogenicity of substantia nigra area $> 0.22\ cm^2$; UMN: upper motor neuron.

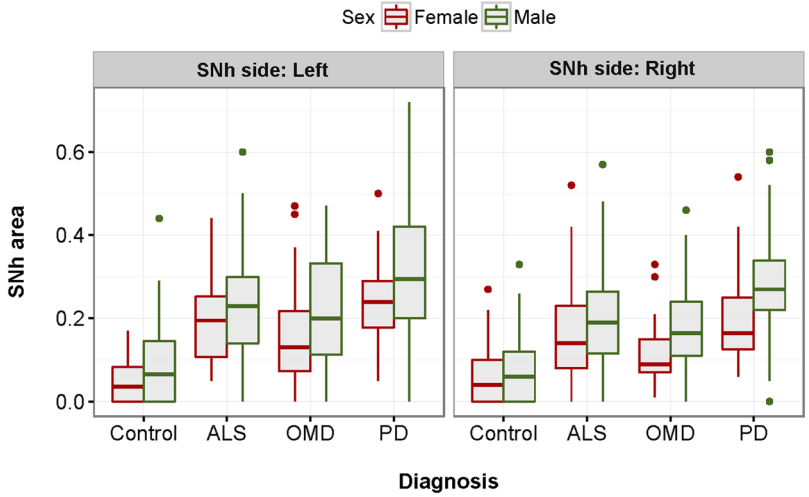

Figure 1. SNh area in different subgroups of patients and controls according to the SNh side and to gender. ALS: amyotrophic lateral sclerosis; OMD: other movements disorders (atypical parkinsonism, vascular parkinsonism and essential tremor); PD: Parkinson's disease; SNh: hyperechogenicity of the substantia nigra.

	Estimate	Lower 0.95	Upper 0.95	p
ALS	0.14	0.11	0.169	< 0.001**
PD	0.196	0.167	0.225	< 0.001**
OMD	0.11	0.078	0.141	< 0.001**
Left SNh	0.029	0.016	0.042	< 0.001**
Male gender	0.047	0.025	0.07	< 0.001**
Age	0.001	0	0.002	0.135

Table 2. Mixed lineal regression model that analyses differences in the SNh area between controls and subgroups of patients. ALS: amyotrophic lateral sclerosis; OMD: other movement disorders (atypical parkinsonism, vascular parkinsonism and essential tremor); PD: Parkinson's disease; SNh: hyperechogenicity of substantia nigra.

SNh could reflect a gender-mediated differential vulnerability to certain diseases. However, this result must be taken cautiously because of the small number of studied PLS patients. Moreover, differences by gender could also be the result of a lower ultrasound penetration rate in women than men.

We found that left SNh side was larger in each cohort of patients and controls, but found no relationship between side of onset or side of symptoms predominance and the area of contralateral SNh. A larger SNh area contralateral to disease predominance has been found in PD[5], but not in ALS[8]. Nor has SNh area been observed to correlate with the degree of clinical or subclinical impairment in PD patients, which suggests that SNh size does not actually reflect the degree of neuronal degeneration[5,29]. Up to now, to the best of our knowledge, no study has compared the right with the left SNh area. However, in those studies reporting SNh sizes on both sides in large cohorts of PD patients and healthy controls, the reported left SNh area has almost always been larger than the right one[24,27,30–32]. It could be argued that side-related variations in the SNh area are explained by changes in the probe placement on the right and left side. Indeed, shifts in the position and angle of the probe can cause variations in the size of midbrain or midbrain structures. However, different studies, with diverse sonographers have found similar results[24,27,30–32]. Moreover, side differences remained stable when accounting for the area of the ipsilateral mesencephalon[31,32], suggesting that they cannot be explained by variations of the imaged midbrain. Finally, previous MRI studies in healthy individuals have also found larger iron deposits in different structures of the left brain hemisphere, including the SN[33,34]. All this suggests that technical issues are not responsible for the differences between left and right sides. The fact that, in our study, this SNh side predominance was found in healthy individuals and patients with different diseases suggests that genetic or constitutional factors (e.g., handedness), but not disease-related factors, could underlie these differences. Interestingly, an increased dopamine metabolism has been found in the left SN[34] and handedness seem to influence the side of onset and predominance of impairment in ALS and PD[35,36]. Considering this and the overall predominance of right-handedness in the population, the left SNh area predominance found in our study could reflect an increased vulnerability of the left-brain hemisphere in right-handed individuals due to an increased dopamine metabolism as previously suggested[33,34], what ultimately results in an asymmetric onset or impairment of the disease. However, further studies in right- and left-handed patients and controls are warranted to test the influence of handedness in SNh.

In the multivariate analysis, we found that fALS, but not history of other neurodegenerative diseases, was associated with a larger SNh area in ALS patients. This effect appeared independent of the identified mutation.

	SN− (n = 42)	SN+(n = 55)
Age (years)		
Mean (*SD*)	57.35 (12.84)	62.68 (11.48)
Median (*IQR*)	57.45 (47.65, 68.2)	62.97 (56.54, 71.55)
Gender (male)		
n (%)	20 (62.5%)	41 (74.5%)
Body mass index, n = 102		
Mean (*SD*)	27.22 (4.49)	27.18 (4.3)
Median (*IQR*)	26.53 (24.54, 29.76)	26.53 (23.98, 29.76)
Family history	7 (16.7%)	18 (32.7%)
Dementia n (%)	1 (3.1%)	8 (14.5%)
PD n (%)	1 (3.1%)	2 (3.6%)
Dementia and PD n (%)	2 (6.2%)	0 (0%)
ALS or FTD n (%)	3 (9.4%)	8 (14.5%)
Region of onset		
Bulbar n (%)	6 (18.8%)	15 (27.3%)
Spinal n (%)	26 (81.2%)	40 (72.7%)
Parkinsonism		
n (%)	1 (2.4%)	3 (5.5%)
Time from onset (months)		
Mean (*SD*)	36.45 (46.83)	32.58 (32.2)
Median (*IQR*)	15.58 (8.43, 55.51)	21.6 (13.62, 42.77)
ALSFRS-R		
Mean (*SD*)	34.06 (8.55)	35.41 (7.53)
Median (*IQR*)	34.5 (31, 40.5)	36.5 (31.25, 41.75)
UMN score		
Mean (*SD*)	5.78 (5.94)	4.75 (5.11)
Median (*IQR*)	3.5 (0, 12.25)	2.5 (1, 9)
EB impairment (n = 80)		
No	21 (72.4%)	30 (58.8%)
Mild	8 (27.6%)	19 (37.3%)
FTD	0 (0%)	2 (3.9%)
Familial or genetic ALS, n (%)	3 (9.37%)	9 (16.36%)
C9ORF72, n (%)	1 (3.12%)	6 (10.91%)
SOD1, n (%)	1 (3.12%)	3 (5.45%)
Unknown, n (%)	1 (3.12%)	0 (0%)
Ferritin ug/L		
Mean (*SD*)	195.31 (155.14)	192.31 (128.31)
Median (IQR)	154 (68, 311)	155 (105.25, 240.25)

Table 3. Demographical, clinical and analytical characteristics of ALS patients classified according to the predefined threshold of SNh area (0.22 cm^2). ALS: amyotrophic lateral sclerosis; BMI: body mass index; EB: executive or behavioural; FTD: frontotemporal dementia; PD: Parkinson's disease; SNh: hyperechogenicity of the substantia nigra; SN+: area of hyperechogenicity of one substantia nigra > 0.22 cm^2; SN−: area of hyperechogenicity of both substantia nigra ≤0.22 cm^2; UMN: upper motor neuron.

In PD, SNh+ has been associated with family history of PD in healthy subjects[24] and has been found in the pre-symptomatic carriers of mutations that cause PD[5]. Furthermore, SNh+ healthy subjects have an increased risk of PD after a 5-year follow-up[37]. Altogether this suggests that SNh is a marker of genetic factors that predispose to PD[5,24]. Our results are consistent with those in PD and suggest that the Mendelian forms of ALS are also predisposed to SNh. Conversely, family history of PD or dementia was not associated with larger SNh in ALS, suggesting that in sALS other non-genetic factors influence the SNh size.

We found no influence of ageing in SNh area. Previous studies in healthy subjects and patients have reported conflicting results[5,24,31,32]. Although SNh can be found even in early childhood[32], it seems that a mild increase in SNh area in both adolescence and very old subjects (>75 years) can occur[5,31,32]. However, during adulthood (30–75 years old) the SNh area remains more or less stable[5,24,31,32], which agrees with our results.

Altered ferritin levels have been found in ALS patients and associated with poor prognosis[38] and could indirectly reflect brain iron deposits. However, we found no correlation between ferritin levels and SNh area. We neither found correlation of executive or behavioural scores with SNh area.

Low premorbid BMI has been associated with a higher risk of ALS, and genetic factors could underlie this relationship[39]. Nevertheless, we found no association between SNh and premorbid BMI either.

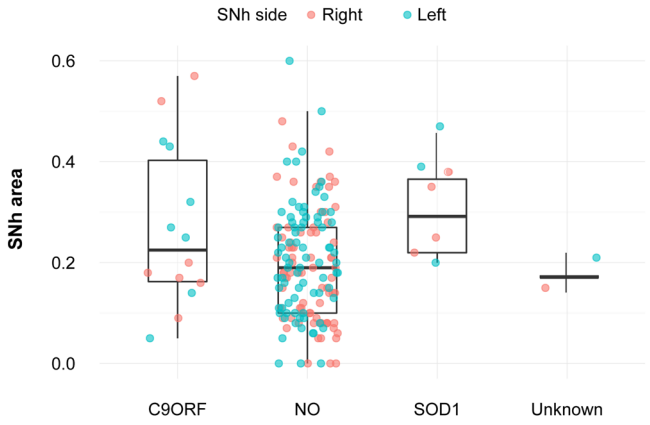

Figure 2. SNh area on the left and the right side in sporadic or familial ALS patients harbouring mutations ("C9ORF72" and "SOD1"), familial ALS without known mutations ("Unknown") and sporadic patients not carrying mutations ("NO"). SNh: hyperechogenicity of the substantia nigra.

	Estimate	Std. Error	Lower 95%	Upper 95%	P-value
PMA	−0.006	0.029	−0.063	0.051	0.831
PLS	−0.067	0.034	−0.135	0.001	0.055
History of PD or dementia	0.006	0.029	−0.05	0.063	0.824
Age	0.001	0.001	0	0.003	0.163
Male gender	0.036	0.023	−0.009	0.082	0.119
fALS	0.083	0.031	0.021	0.145	0.01*
Left SNh	0.022	0.012	−0.001	0.045	0.066

Tablee 4. Mixed lineal regression model that analyses the factors that contribute to the SNh area. fALS: familial ALS; PLS: primary lateral sclerosis; PMA: progressive muscular atrophy; PD: Parkinson's disease; SNh: hyperechogenicity of substantia nigra.

Finally, no association between the clinical variables (ALSFRS-R, disease duration, cognitive or behavioural impairment or parkinsonism) and SNh area was found, although very few patients exhibited parkinsonism and this result must be taken cautiously. Our results agree with previous reports that have found no association between SNh or SNh+ and ALSFRS-R, disease duration or ALS subtype (bulbar or spinal)[6–9]. Similarly, in PD, most studies have observed no association between SNh and clinical variables[5].

Strengths and limitations. Our study represents the largest and most thorough study of SNh in different neurodegenerative diseases and in controls conducted to date. We studied phenotypes (PMA and PLS) and variables that have not been analysed before. We also introduced several methodological improvements compared to previous studies. Firstly, most reports consider SNh to be a dichotomous variable (SNh+ and SNh−). Despite SNh+ being arbitrarily defined (percentile 90 of healthy subjects), it can be useful as a diagnostic biomarker. However, when the aim is to study the factors that contribute to SNh, dichotomising a biological variable that is in fact continuous is not methodologically sound. Secondly, in order to increase the statistical power and to avoid unnecessary data loss, we included data of both SNh sides in the inferential analysis. To date, all studies have either considered only the biggest SNh or analysed the mean SNh, which does not reflect the anatomic basis. Our data show that SNh is unequally distributed between sides. Consequently, not considering this item in inferential studies can be a source of bias. Thirdly, previous reports had studied the correlation of clinical variables and SNh in ALS, but had not accounted for confounding factors. We performed a multivariate analysis and, consequently, our results are more reliable.

However, our study also has some limitations. Firstly, the sample size for some studied variables (fALS, PLS phenotype or parkinsonism) was small as their prevalence is low, so the results on these items must be taken cautiously. Secondly, no sample size calculation was made and the overall number of included ALS patients could be insufficient for the number of studied variables. This could have limited the statistical power in the multivariate analysis and, consequently, some of the variables that show an association trend could also have some influence on SNh. Thirdly, the medical records of the ALS patients' relatives were not systematically reviewed. This could introduce a recall bias since patients could be more likely to become aware of a family history of ALS than one of other neurodegenerative diseases. Therefore, the influence of family history of dementia and PD could have been underestimated herein. However, these limitations do not invalidate the positive results.

Conclusions

We show that left side and male gender are risk factors for SNh in both controls and different diseases. We report for the first time that fALS and ALS-causing mutations (*C9ORF72* and *SOD1*), but not other disease-related variables, are major contributors to SNh. Finally, we demonstrate that SNh can also be found in PMA, and less frequently in PLS. Although the number of fALS, PMA and PLS patients included was small, based on our results and previous reports in both patients and healthy subjects, we hypothesise that SNh is a marker of some constitutional (male gender, handedness) or genetic vulnerability factors for neuronal degeneration that extend beyond the nigrostriatal system. Remarkably, reduced intracortical inhibition within the motor cortex, as assessed by transcranial magnetic stimulation, which is an early feature in ALS and PD[40], has also been reported in healthy subjects with SNh[41], further supporting this vulnerability hypothesis. Larger studies in both healthy populations and presymptomatic individuals and patients who carry ALS mutations are warranted to confirm our results and hypotheses.

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Author Contributions

J.F.V.C. designed the study, participated in clinical data acquisition and interpretation, and wrote and edited the manuscript. J.I.T., L.M.C. and G.F. participated in ultrasound data acquisition and critically revised the manuscript. V.F.F. participated in the study design and interpretation and critically revised the manuscript. J.P.T. and F.C. performed genetic analysis and critically revised the manuscript. T.S. designed and supervised the study and critically revised the manuscript. J.F.V.C. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have approved the submitted version of the paper.

Additional Information

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Brain signal intensity changes as biomarkers in amyotrophic lateral sclerosis

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Introduction

Amyotrophic lateral sclerosis (ALS) is characterised by a progressive upper (UMN) and lower motor neuron (LMN) impairment. Clinical LMN signs can be elicited early in the disease. Moreover, electromyography (EMG) detects LMN impairment before it is clinically evident and, consequently, has been incorporated to the ALS diagnostic criteria.[1] However, clinical UMN signs are not always appreciable, since they may be obscured by the LMN impairment. Several UMN biomarkers have been proposed in recent years. However, they have not been translated into clinical practice because they are difficult to implement, or because results cannot be individually interpreted.[2] Therefore, biomarkers of UMN impairment that are reliable and easy to assess are a priority for both research and clinical practice.

Iron-related hypointensities, particularly in the motor cortex (IRhMC), have been described in T2-weighted magnetic resonance (MR) images in ALS patients for more than 20 years.[3] Recent technical advances in MR imaging, such as susceptibility-weighted (SW) imaging at higher magnetic fields, have improved the detection of IRhMC in recent years.[4,5] This hypointense rim seems to correspond to iron bound to ferritin inside activated microglia in the deep cortical layers of the motor cortex,[4–6] and seems to correlate with degree of UMN impairment.[5,7,8] However, precentral gyrus hypointensities have also been described in increasing ageing healthy controls.[9,10]

Hyperintensities at different levels of corticospinal tracts (HCT), particularly in the FLAIR sequence, have been described in ALS patients and also, to a lesser extent, in healthy controls.[2,11] They might reflect the degeneration of corticospinal tracts, albeit they were poorly correlated with clinical UMN signs in previous studies.[2,11]

The exact cause and meaning of both MR changes in patients and controls remains unknown as most published case series are short and available clinical data are scarce. In this study, we aimed to study the contribution of demographical, clinical, analytical and genetic factors to these MR findings in both ASL patients and healthy controls to analyse their role as surrogate biomarkers of UMN impairment.

Methods

Subjects and definitions

For this study, patients clinically diagnosed with ALS,[12] who visited our ALS Unit between November 2013 and September 2016, were recruited.

As healthy controls, subjects with no neurological disease and with a normal neurological examination were enrolled. We also prospectively included those patients in whom an ALS diagnosis was suspected during the recruitment period, but were finally diagnosed with other diseases (ALS mimics).[13]

Patients and controls were evaluated by the same neurologist, and demographical and clinical data were prospectively recorded in a database.

Family history and genetic analysis

ALS patients were systematically asked for a family history of ALS or frontotemporal dementia (FTD) and they were subsequently categorised as familial ALS (fALS) or sporadic ALS (sALS).[14] Both fALS and sALS patients were screened for *C9orf72* with repeat primed PCR, as previously reported.[15] The *SOD1* gene was sequenced in the fALS patients who did not carry the *C9orf72* expansion. Finally, both the fALS and sALS patients who carried *C9orf72* mutations were considered genetic ALS.

Clinical and analytical variables

Premorbid body mass index (BMI) was recorded per patient. Patients were categorised in phenotypes according to degree of UMN and LMN impairment, as previously defined:[12,16] classical ALS (cALS), LMN predominant ALS (LMN-ALS) and UMN predominant ALS (UMN-ALS). Age, gender, dates of symptom onset and of diagnosis, and site and side of symptoms onset (or side predominance in bulbar onset cases), were recorded for all the ALS patients.

Upon recruitment, ALS patients were examined and the ALSFRS-R score[17] and number of brisk reflexes (UMN score) for a maximum of 16 were recorded. Levels of ferritin were obtained in 83 patients by immunoturbidimetry[18] using a Beckman Coulter AU 5400 following the manufacturer's instructions.

MR imaging acquisition and analysis

MR imaging examinations were performed in a 3T scanner (Signa HDxt, GE Healthcare, Milwaukee, USA) using a transmit-receive head coil array with eight elements. MR

images were visually assessed by two neuroradiologists (MM and JCP) blinded to the diagnosis. IRhMC on the SW images were assessed on the posterior bank of the motor cortex on the left and right hemispheres. Following the clinical categorisation of ALS, the motor cortex was subdivided into three subregions, which corresponded to the cortical representation of lower limbs, upper limbs and bulbar musculature. [19–23]Since iron-related changes differ as far as their intensity and extent are concerned,[4,24] a scoring system that accounted for these two characteristics was designed. as: 0, normal intensity; 1, mildly hypointense, similar signal intensity to corpus callosum; 2, markedly hypointense, similar signal intensity to subependymal veins.

The HCT score took into account two anatomic regions of the corticospinal tract on FLAIR: the subcortical precentral white matter (SPWM) and the posterior limb of the internal capsule (IC). For each anatomic region, the following scores were assigned depending on their signal intensity: 0, normal intensity; 1, mildly hyperintense, similar signal intensity to the cortex; 2, markedly hyperintense, superior signal intensity to the cortex. The right and left sides were analysed separately (Figure 1 D and E).

Further details on the image acquisition and analysis can be found as supplementary material.

Statistical analysis

Data were summarised by the following descriptors: mean, standard deviation, median, and first and third quartiles for the continuous variables; relative and absolute frequencies for the categorical variables. The inter-observer agreement for MSS and HCT score were calculated with the weighted κ. Heat maps were used to evaluate the effect of the symptom onset site on the MSS. A Wilcoxon test was ran to assess the effect of side of disease onset on the MSS. Associations of the demographical and clinical variables with the MSS and the HCT score in both patients and controls were assessed by Spearman's correlations and mixed linear regression models. In these models, it was assumed that the MRI score depended on fixed effects (covariates) and random effects due to inter-individual variability. A non-linear association of premorbid BMI with the MSS was included in the model by means of natural splines. [25] All the statistical analyses and graphs were performed using the R software (version 3.2.2).

Ethical approval

The study was approved by the Ethics Committee for biomedical research of the La Fe Hospital. All the participants gave written informed consent.

Results

Population characteristics and image analysis

The study included 102 ALS patients, 28 ALS mimics and 20 healthy controls. ALS patients were older and more frequently male than the controls. Most patients were in an early disease stage, and a short time had elapsed since the diagnosis was made. Eight were classified as fALS. A pathologic expansion in *C9orf72* was found in seven fALS patients (87.5%) and a SOD1 mutation (p.N139H) in the remaining fALS patient (12.5%). Two sALS patients (2.13%) carried pathologic expansions in *C9orf72*.

The inter-observer agreement was good for the MSS (κ = 0.77 [0.73, 0.82]) and only fair for the HCT score (κ = 0.46 [0.40, 0.52]). ALS patients showed higher MSS and HCT scores than controls. The demographical, clinical and radiological characteristics of both patients and controls are summarised in Table 1.

Contribution of demographical variables to the visual scores in the controls

Older age, but not gender, was associated with a higher MSS (OR=1.106 [1.033, 1.212], p=0.011; and OR=2.142 [0.314, 19.379], p=0.45) in the controls. However, there was no evidence for an association between the HCT score and age/gender (OR=1.022 [0.988, 1.059], p=0.221; and OR=2.177 [0.717, 6.907], p=0.175).

Visual scores in the ALS patients

The cALS and UMN-ALS patients showed a similar MSS, but a higher one than the LMN-ALS patients after adjusting for age and gender (OR=0.168 [0.072, 0.377], p<0.001) (Figure 2A). However, the cALS patients presented higher HCT scores than both the UMN-ALS and LMN-ALS patients after adjusting for age and gender (OR=0.239 [0.072, 0.757], p=0.016; and OR=0.221 [0.094, 0.507], p<0.001) (Figure 2B).

Patients showed greater iron-related hypointensities in those regions of the motor cortex that corresponded with the region of symptoms onset (bulbar, upper limb and lower limb). However, no association for side of symptoms onset was found with side of

the motor score (p=0.26 and p=0.67 for right and left side onsets) or for HCT score predominance (p=0.52 and p=0.85 for right and left side onsets) (Supp. Figure 1A and 1B). Both the MSS (Figure 3) and HCT score were frequently symmetrical.

Visual scores in genetic vs. non-genetic ALS

The median MSS and HCT scores were similar in the genetic ALS (5.5 [2.88, 6.38] and 4 [4, 4.75] respectively) than in the non-genetic ALS patients (5 [0, 8.62] and 4 [2, 6] respectively) (Supp. Figure 2A and 2B).

Contribution of clinical variables to the visual scores in the ALS patients

Figure 4 represents correlations among both visual scores and between them and the clinical variables in the ALS patients. A strong correlation between the MSS and the HCT score ($r = 0.76$, $p < 0.001$) was found. UMNs and ALSFRS-R correlated weakly with the HCT score ($r = 0.29$, $p<0.001$; $r = -0.22$, $p=0.008$) and more strongly with the MSS ($r = 0.46$, $p < 0.001$; and $r = -0.34$, $p < 0.001$). Similarly, the disease progression rate correlated weakly with the MSS and HCTs ($r = 0.26$, $p=0.004$; $r = 0.29$, $p=0.001$). Finally, a weak correlation between age and the MSS ($r = 0.24$, $p=0.002$) and a weak inverse correlation between disease duration and the HCT score ($r = -0.19$, $p=0.023$) were found.

For the multivariable analysis, these clinical variables (spinal *vs.* bulbar onset, UMN score and ALSFRS-R) were selected according to previous literature and the results of the correlations, which were most probably associated with the visual scores. We also adjusted for age and gender. We included two more variables for the MSS variables: serum ferritin levels since increased serum ferritin has been found in ALS patients[26] and could reflect brain ferritin deposits; premorbid BMI since a higher BMI has been associated with larger iron deposits in the cortex of healthy controls.[27] Bulbar onset and higher UMN scores were associated with a higher MSS and HCTs (Table 2 and Table 3). No other statistically significant association was found, albeit a possible non-linear association of premorbid BMI with a higher MSS (Table 2, Figure 5).

Discussion

IRhMC in controls and ALS patients

Iron plays a fundamental role in many biological processes in the brain and has been shown to accumulate in different brain regions in healthy ageing individuals.[28] In several

neurodegenerative diseases, iron accumulates in specific brain regions in larger amounts than reported in healthy individuals, and is thought to induce oxidative stress.[28] Due to its magnetic properties, magnetic susceptibility variations due to brain iron deposition can be detected and quantified in MR imaging.[9,29] Iron deposits in the cortical grey matter of healthy individuals have been shown to increase with age, especially in the motor cortex.[9] Several quantitative assessment methods have been developed to measure the magnetic susceptibility changes in MR images, but require post-processing.[9,30,31] However, these changes are also visible as hypointensities in different sequences (T2, FLAIR, T2* and SW images) can be easily assessed qualitatively, and have been proven reliable and accurate.[31] These hypointensities have been described particularly in the motor cortex (IRhMC) of ALS patients and have attributed to iron deposits.[5] However, they are rarely found in healthy individuals or in other cortical areas.[30]

In this study, IRhMC were qualitatively assessed in ALS patients and controls. Age was associated with larger iron deposits in controls, but not in ALS patients, which agrees with previous studies.[4,9,27] This increase in iron deposits with ageing controls seems to correspond to transferrin and ferritin accumulation in astrocytes and microglia.[10] Conversely in ALS patients, they have been attributed to ferritin loads inside activated microglia and are believed to be markers of UMN degeneration.[4–6,8,32] In our study, IRhMC were strongly influenced by disease phenotype (degree of UMN impairment and bulbar onset), which probably limited the effect of age. Although a correlation was found between disability and IRhMC, as in previous reports[5,24], this association was lost in the multivariable analysis, where UMNs were included as a covariate. This suggests that the correlation of disability with IRhMC can actually be explained by UMN impairment.

Our data interestingly suggest that premorbid BMI can influence IRhMC in ALS patients, independently of UMN signs and disability. This association has been previously found in a large cohort of healthy individuals[27], and suggests a non-disease-related effect. How BMI can influence cortical brain iron deposits, and whether this relates to UMN degeneration or not, deserve further research.

We found no differences in iron deposits between genetic ALS and non-genetic ALS, which suggests that, unlike *substantia nigra* hyperechogenicity[33] (manuscript submitted for publication), genetic factors do not influence IRhMC. [34–37]

Nor did we find any association between side of disease onset and side of IRh predominance as IRhMC was almost always found bilaterally and symmetrically. This indicates that the spread of the disease to the contralateral hemisphere, probably through the corpus callosum, occurs early in the disease before it spreads to other motor homunculus regions. Finally, serum ferritin has not been associated with brain iron deposits in ALS patients, which suggests that iron metabolism is not related with these deposits according to previous findings in healthy individuals[27] and ALS patients.[32]

HCT in controls and ALS patients

The HCT is a sign that can be detected especially in FLAIR images and at different CT levels of both ALS patients and controls. It is thought to reflect areas of reduced axonal and myelin density within the CT[38], but its meaning could differ depending on the subject and the level of the CT where it is found. For example in healthy subjects, hyperintensity is frequently found at the IC, which probably reflects areas of the CT with thicker fibres and lower density.[38] Conversely at the SPWM, hyperintensity appears in healthy subjects, but only with increasing age,[39] which probably reflects some degree of Wallerian degeneration. In ALS patients, the HCT probably reflects areas of Wallerian degeneration,[38] especially at the SPWM, and is taken as a more specific sign than when found at the IC.[11] Our HCT score accounted for both levels of the CT, which might explain that we failed to find an association with age in healthy controls. Only weak correlations between HCTs and clinical variables were found in the ALS patients in our study. However, the HCT score in the multivariable analysis was associated with UMN signs and bulbar onset. A previous study failed to find a correlation between the HCT and clinical UMN signs,[11] but it did not perform a multivariable analysis. Finally as with IRhMC, we found similar HTCs in genetic vs. non-genetic ALS patients.

Meaning of IRhMC and the HCT: similarities and discrepancies

Our study data support the hypothesis that both IRhMC and the HCT are markers of different pathological events (neuronal soma and axonal degeneration, respectively) of

the same UMN degeneration process. We found a strong correlation between them; both were most frequently symmetrical and were associated with the same clinical factors (UMN impairment and bulbar onset). Interestingly, previous studies have shown that both UMN signs[40] and white matter degeneration measured with DTI[2,41] are more severe in bulbar onset than in spinal onset patients. Together with our results, this suggests that bulbar onset favours UMN degeneration and spread.

Despite these similarities, it is worth highlighting one difference. The HCT score (but not the MSS) was lower in the UMN-ALS than in the c-ALS phenotype. This finding was unexpected if we consider that both scores are associated with UMN signs. However, UMN-ALS has a more benign course than c-ALS.[16] Therefore, these differences in scores could actually reflect that despite both phenotypes exhibiting similar degeneration of the neuronal soma, Wallerian degeneration develops later in UMN-ALS patients.

Overall, the MSS showed a better reproducibility and a stronger association with the clinical variables than the HCT. We also found an association between site of symptoms onset and the motor homunculus region with predominant iron deposits. Moreover, the anatomically most distant region to that of the clinical onset was the one with lower scores. This is in line with previous studies, which have shown that motor neuron degeneration is a "focal process that advances contiguously, summates over time, and creates graded loss".[40,42] These data favour the use of IRhMC *vs*. the HCT as a marker of UMN degeneration in ALS patients.

Strengths and limitations

This is the largest and most thorough study into the factors that contribute to brain signal intensity changes in 3T magnets in ALS patients. Compared to previous studies, several methodological improvements were included. We firstly accounted for confounding variables when analysing the association of clinical variables and the studied MRI signs. Secondly, we recruited a clinic-based cohort of ALS patients, regardless of them showing UMN signs or not. Thirdly, we measured the IRhMC in three subregions according to the pathophysiological spread of disease.

However, our study also has some limitations. First of all, the sample size for genetic ALS is small, and the results obtained about this item must be taken cautiously. Secondly,

the study design allows for association studies that provide information about the meaning of these signs, although their role as diagnostic, progression or prognostic biomarkers should be analysed in future works. Thirdly, we assessed both scores qualitatively. However, a qualitative assessment has proven reliable and accurate [31], and we found good inter-rater reproducibility.

Conclusions

HCT and especially IRhMC are reliable markers of UMN degeneration in ALS patients and associate with bulbar onset, but none is genetically determined. Moreover, age and BMI should be considered to be potential modifiers of IRHMC. A regional analysis of IRHMC following the motor homunculus could be an easy reliable method to quantify and monitor UMN loss.

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	Non-genetic ALS (n = 92)	Genetic ALS (n = 10)	ALS mimics (n = 28)	Healthy controls (n = 20)
Age (years) *Mean (SD)* *Median (IQR)*	61.34 (12.09) 62.64 (55.35, 70.21)	60.07 (10.86) 62.41 (54.15, 68.7)	55.26 (17.34) 55.83 (40.88, 66.61)	51.69 (15.26) 48.77 (40.9, 57.39)
Gender (male) n (%)	59 (64.13%)	6 (60%)	15 (53.6%)	8 (40%)
Region of onset Bulbar n (%) Spinal n (%) Axial n (%) Generalised n (%) Proximal UL n (%) Hand n (%) Lower limb n (%)	23 (25%) 76 (75%) 2 (2.17%) 4 (4.35%) 6 (6.52%) 19 (20.65%) 38 (41.3%)	3 (30%) 7 (70%) 0 (0%) 0 (0%) 0 (0%) 3 (30%) 4 (40%)		
Phenotype cALS n (%) LMN-ALS n (%) UMN-ALS n (%)	51 (55.43%) 31 (33.7%) 10 (10.87%)	6 (60%) 3 (30%) 1 (10%)		
Side of onset or side predominance, n=99 Right n (%) Left n (%) Symmetric n (%)	39 (43.82%) 38 (42.7%) 12 (13.48%)	4 (40%) 6 (60%) 0 (0%)		
Time from onset (months) *Mean (SD)* *Median (IQR)*	26.22 (35.9) 14.78 (9.38, 25.54)	11.79 (4.4) 13.32 (8.91, 14.78)		
Time from diagnosis (months)	11.4 (32.25) 2.25 (1.19, 7.87)	1.69 (1.52) 1.75 (0.22, 2.72)		

Mean (SD) *Median (IQR)*				
ALSFRS-R *Mean (SD)* *Median (IQR)*	37.38 (6.31) 38.5 (34.75, 42)	39.8 (6.3) 41.5 (41, 42.75)		
Clinical staging 1 (before diagnosis) 2A (diagnosis) 2B (two regions) 3 (three regions) 4 (NIV or gastrostomy)	 8 (8.7%) 31 (33.7%) 21 (22.83%) 25 (27.17%) 7 (7.61%)	 0 (0%) 4 (40%) 5 (50%) 1 (10%) 0 (0%)		
UMN score *Mean (SD)* *Median (IQR)*	4.88 (4.84) 3 (0, 9)	4.6 (5.36) 2.5 (1, 7.5)		
Motor sum score *Median (IQR)*	5 (1.5, 8.62)	5.5 (3.75, 7.12)	0 (0,0)	0 (0,0)
HCT score *Median (IQR)*	4 (2, 6)	4 (4, 4.75)	2 (0, 2)	2 (0, 2)

Table 1. Demographical, clinical and radiological characteristics of ALS patients and controls. ALS: amyotrophic lateral sclerosis; ALSFRS-R: ALS functional rating scale (revised version); cALS: classical ALS; IQR: interquartile rank; HCT: hyperintensities of the corticospinal tract; LMN: lower motor neuron; LMN-ALS: lower motor neuron predominant ALS; NIV: non-invasive ventilation; UL: upper limb; UMN: upper motor neuron; UMN-ALS: upper motor neuron predominant ALS.

	Estimate	SE	exp(Estimate)	Lower 95%	Upper 95%	P-value
Age	0.024	0.018	1.024	0.989	1.062	0.189
Male gender	-0.697	0.44	0.498	0.208	1.176	0.113
UMN score	0.3	0.052	1.35	1.222	1.502	<0.001**
Bulbar onset	1.406	0.473	4.08	1.626	10.459	0.003**
ALSFRS-R	0.049	0.037	1.05	0.977	1.132	0.195
Log(Ferritin)	-0.292	0.291	0.747	0.422	1.325	0.316
pBMI1	0.35	0.994	1.419	0.208	10.672	0.725
pBMI2	6.361	3.483	578.911	1.043	946090.232	0.068
pBMI3	2.195	1.311	8.982	0.683	128.215	0.094

Table 2. Mixed lineal regression model that analysed the factors which contributed to the MSS. MSS: motor sum score; pBMI: premorbid body mass index; SE: standard error; UMN: upper motor neuron.

	Estimate	Std. Error	exp(Estimate)	Lower 95%	Upper 95%	P-value
Age	-0.013	0.016	0.987	0.956	1.019	0.421
Male gender	-0.326	0.378	0.721	0.342	1.51	0.387
UMN score	0.143	0.043	1.154	1.062	1.258	0.001**
ALSFRS-R	0.019	0.031	1.02	0.96	1.083	0.525
Bulbar onset	0.886	0.418	2.426	1.076	5.566	0.034*

Table 3. Mixed lineal regression model that analysed the factors, which contributed to the HCT score. HCT: hyperintesity of the corticospinal tracts; SE: standard error; UMN: upper motor neuron.

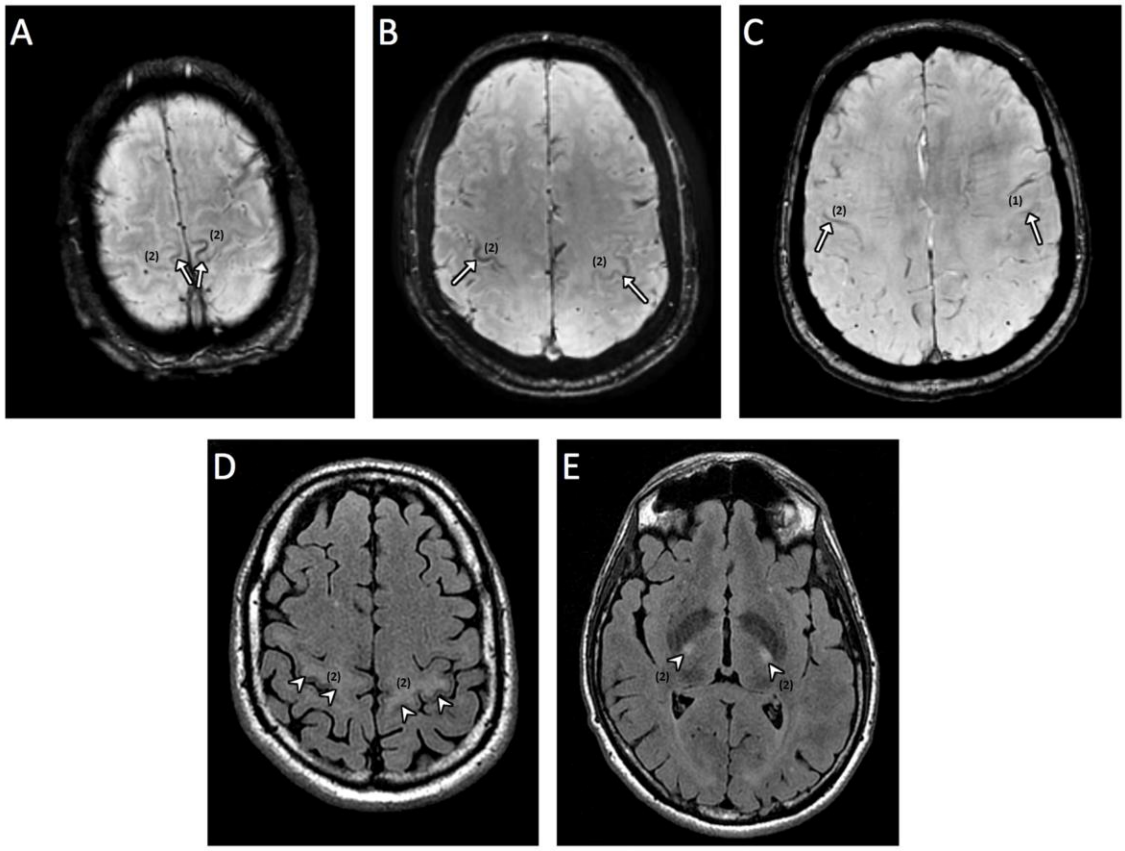


Figure 1. Iron-related hypointensities in the SW imaging of the lower limbs (A), upper limbs (B) and bulbar region (C) of the motor cortex. Hyperintensities of the corticospinal tract on FLAIR in subcortical white matter (D) and capsula interna (E). The numbers in brackets represent the assigned scores in each region and hemisphere.

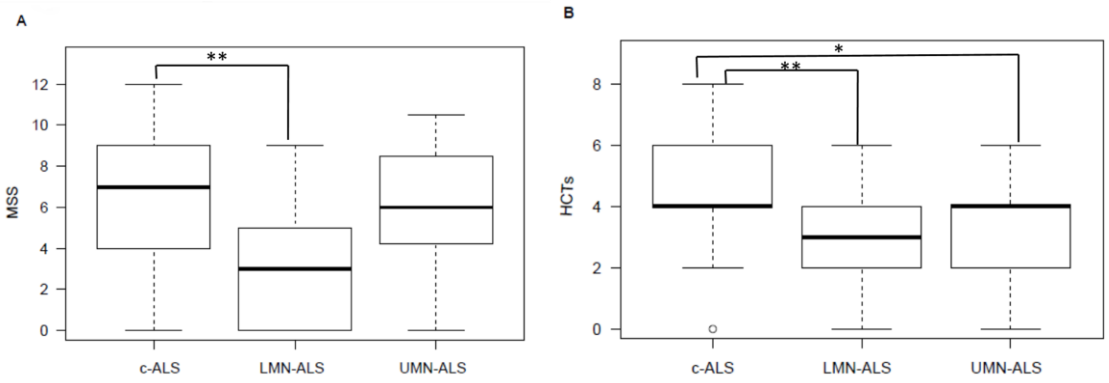


Figure 2. Graphical representation of the MSS (A) and HCTs (B) according to the ALS phenotypes (c-ALS, LMN-ALS and UMN-ALS). c-ALS: classical ALS; LMN-ALS: lower motor neuron predominant ALS; HCTs: hyperintensity of the corticospinal tract score; MSS: the motor sum score; UMN-ALS: upper motor neuron predominant ALS.

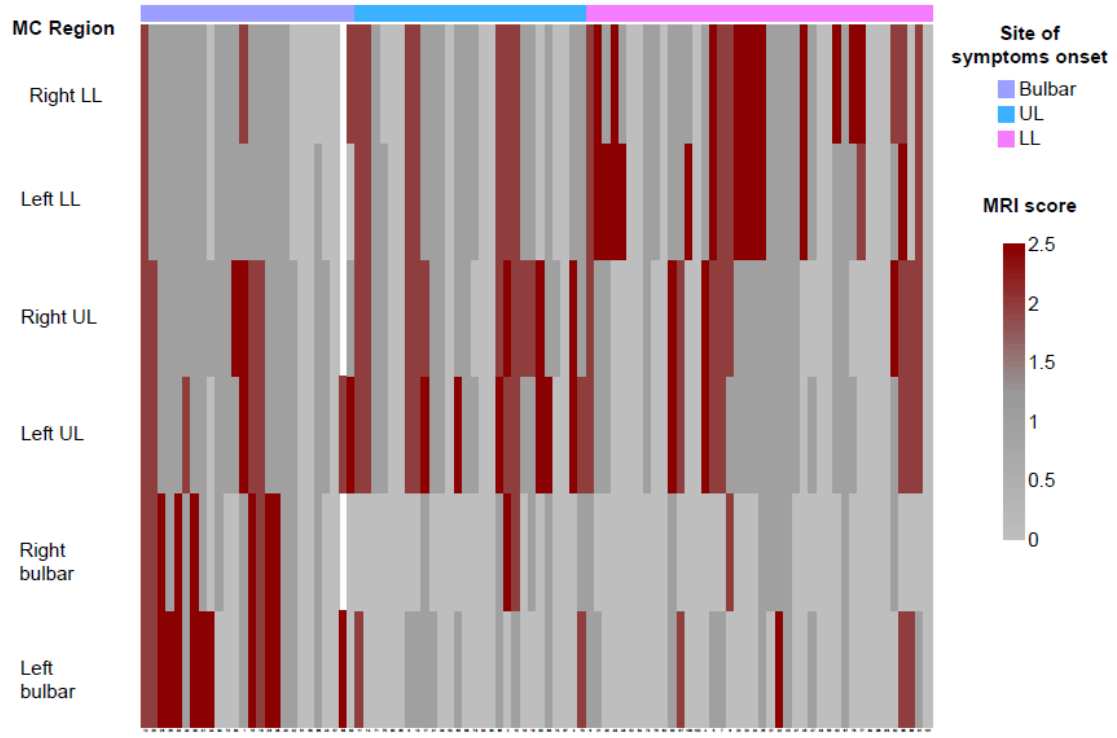


Figure 3. Heat map that represents the iron-related hypointensities scores in the bulbar, UL and LL regions of the motor homunculus in each hemisphere (right or left) according to site of symptoms onset. Patients with bulbar onset obtain higher motor scores in the bulbar regions, followed by the UL regions; the patients with onset in UL obtained higher motor scores in the UL regions, followed by the LL regions; the patients with onset in LL obtained higher motor scores in the LL regions, followed by the UL regions. LL: lower limbs; MC: motor cortex; UL: upper limbs.

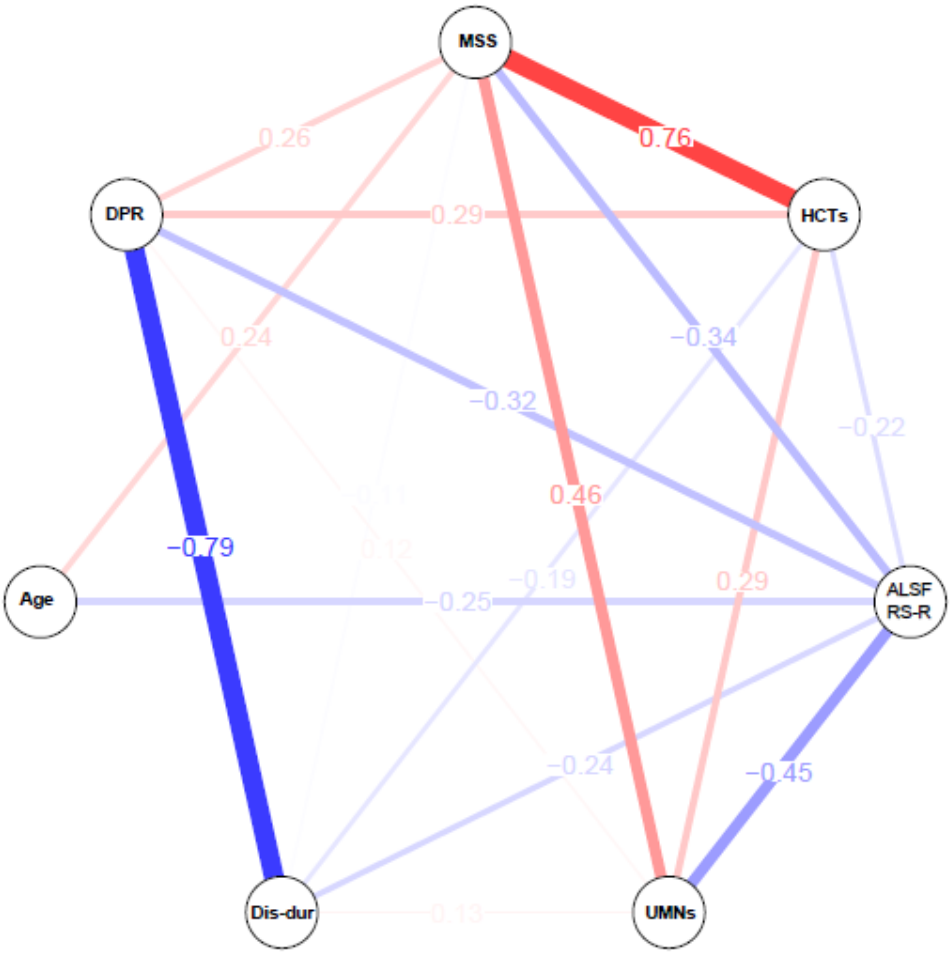


Figure 4. Correlation network between the visual scores (MSS and HCTs) and the variables. Dis-dur: disease duration; DPR: disease progression rate; HCTs: hyperintensity of the corticospinal tract score; MSS: the motor sum score; UMNs: the upper motor neuron score.

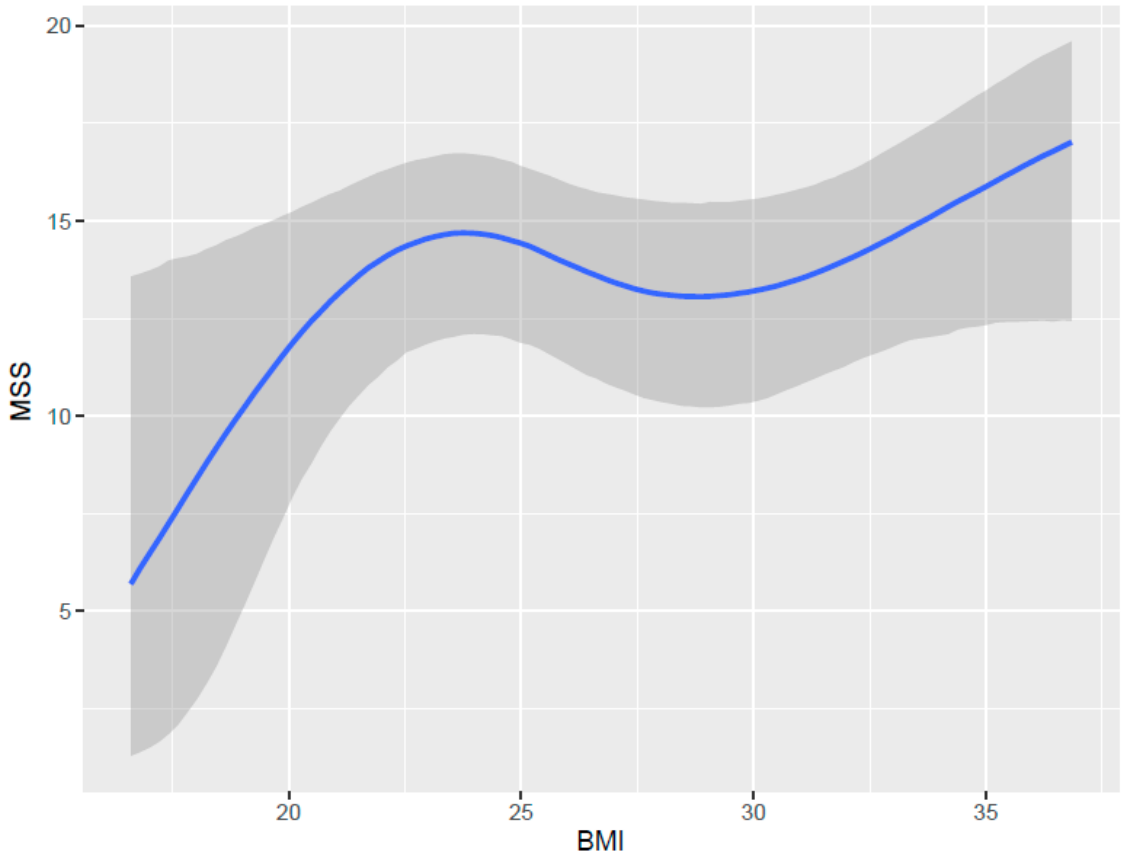
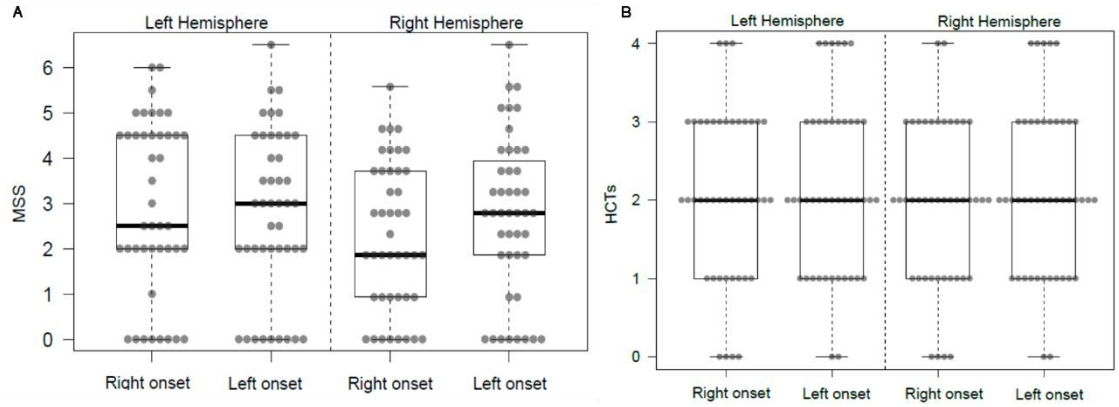
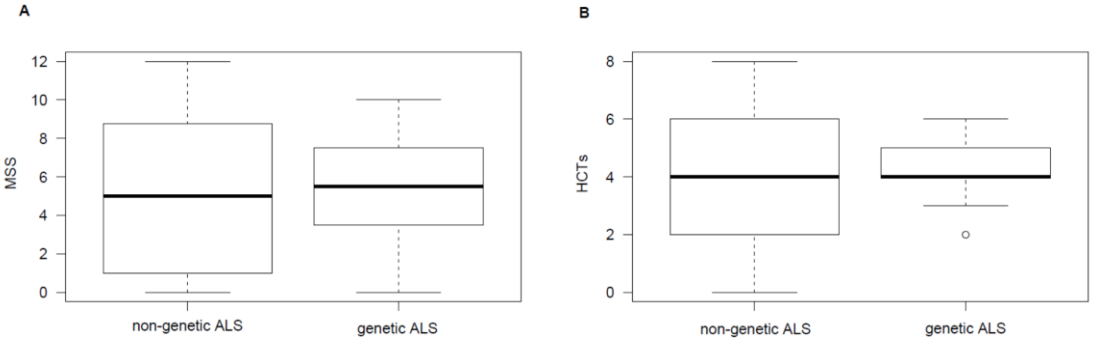


Figure 5. The graphical representation of the contribution of premorbid BMI (pBMI) to IRHMC according to the multivariable model shows a non-linear relationship between the MSS and pBMI, where pBMI<22.5 associates with a lower MSS, and pBMI>30 with a higher MSS. The pBMI between 22.5 and 30 does not seem to influence the MSSIRHMC: iron-related hypointensities in the motor cortex; MSS: the motor sum score.



Supplementary Figure 1. Graphical representation of the right and left MSS (A) and HCTs (B) according to side of disease onset. A trend towards a higher MSS in the left brain hemisphere is seen, regardless of side of onset, and albeit it not being statistically significant. No difference in the HCTs between hemispheres according to side of onset is noted.



Supplementary Figure 2. Graphical representation of the MSS (A) and HCTs (B) in the sporadic and familial ALS patients.

F. VÁZQUEZ-COSTA JF, MARTÍNEZ-MOLINA M, FERNÁNDEZ-POLO M, FORNÉS-FERRER V, FRASQUET-CARRERA M, SEVILLA-MANTECÓN T. ANALYSIS OF THE DIAGNOSTIC PATHWAY AND DELAY IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN THE VALENCIAN COMMUNITY. NEUROLOGIA. 2018; *IN PRESS*.

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Análisis del trayecto y retraso diagnóstico de los pacientes de esclerosis lateral amiotrófica en la Comunidad Valenciana

Analysis of the pathway and diagnostic delay of amyotrophic lateral sclerosis patients in Valencian Community

Resumen

Introducción

La esclerosis lateral amiotrófica (ELA) es una enfermedad insidiosa y clínicamente heterogénea, lo que resulta en un retraso diagnóstico de unos 12 meses. En España el trayecto diagnóstico no ha sido analizado.

Métodos

Se recogieron variables relativas al trayecto y retraso diagnóstico de pacientes diagnosticados de ELA entre octubre de 2013 y julio de 2017.

Resultados

Se incluyeron 143 pacientes de ELA (57% varones, 68% de inicio espinal). El 86% fueron estudiados en centros públicos y un 14% en privados. El retraso diagnóstico medio fue de 13,1 meses (mediana 11.7). El paciente tardó de media 7,9 meses en llegar al neurólogo y éste, 5,2 meses más en diagnosticarle. En la mitad de los pacientes se realizaron pruebas innecesarias y más de un estudio electrofisiológico para llegar al diagnóstico. El retraso diagnóstico fue mayor en los casos espinales ($p = 0,008$), atribuible a los pacientes cuyos síntomas se iniciaron en miembros inferiores, pero sin diferencias entre el sistema público y privado ($p = 0,897$).

Conclusiones

El retraso diagnóstico de la ELA en nuestro medio es similar al de países de nuestro entorno y parece determinado por factores propios de la enfermedad e independiente del sistema sanitario. Las formas de inicio en miembros inferiores constituyen el mayor reto. Los errores diagnósticos del neurólogo son frecuentes y en parte atribuibles a una mala orientación o interpretación del estudio electrofisiológico. La formación específica del neurólogo y

neurofisiólogo general y la derivación precoz a centros de referencia podrían ayudar a reducir la demora.

Abstract

Introduction

Amyotrophic lateral sclerosis (ALS) is a clinically heterogeneous neurodegenerative disease, where an early diagnosis is challenging. Diagnostic delay in different countries is of about 12 months, but has not been studied yet in Spain.

Methods

The diagnostic pathway was reviewed and recorded in ALS patients diagnosed between October 2013 and July 2017.

Results

The study included 143 ALS patients (57% male, 68% spinal onset). Patients were diagnosed either in public (86%) or in private (14%) healthcare centers.

Mean diagnostic delay was 13.1 months (median 11.7). Patients were visited by neurologists 7.9 months after symptoms onset, whereas neurologists needed 5.2 months to reach the diagnosis. In half of the patients, unnecessary diagnostic tests and several electrophysiological studies were performed before reaching the diagnosis. Diagnostic delay was longer in spinal onset cases (p=0.008), due to lower limb onset patients. No differences were found between public and private healthcare system.

Conclusions

The diagnostic delay in ALS in our country is similar to other neighboring countries and seems to depend on disease related factors, but not on the healthcare system. Lower limb onset patients are the greatest diagnostic challenge. Diagnostic mistakes are frequent and partly attributable to an erroneous interpretation of the electrodiagnostic studies. Training programs for general neurologists and neurophysiologists and the early referral to reference centers could help to reduce the diagnostic delay.

Palabras clave: Esclerosis Lateral Amiotrófica, trayecto diagnóstico, retraso diagnóstico, estudio electrofisiológico

Keywords: Amyotrophic lateral sclerosis, diagnostic pathway, diagnostic delay, electrophysiological study

Introducción

La esclerosis lateral amiotrófica (ELA) es una enfermedad neurodegenerativa caracterizada por una debilidad progresiva secundaria a la muerte de motoneuronas corticales y espinales, que típicamente conduce al fallecimiento por insuficiencia respiratoria transcurridos 3-4 años del inicio de los síntomas1.

A pesar de su rápida progresión, el diagnóstico de la enfermedad en fases iniciales ofrece serias dificultades dado su inicio insidioso, su gran heterogeneidad clínica y la ausencia de marcadores diagnósticos2. Esto se traduce con frecuencia en errores diagnósticos, pruebas innecesarias y un retraso diagnóstico de alrededor de un año3–13, lo que representa aproximadamente un tercio de la supervivencia global de la enfermedad. Este retraso diagnóstico parece independiente del país y su sistema de salud2–10,14 y pese a diversas estrategias11 y a las actualizaciones propuestas a los criterios diagnósticos de El Escorial2,16, no se ha modificado significativamente en los últimos 20 años.

En una enfermedad devastadora como la ELA, acortar el tiempo hasta el diagnóstico puede repercutir positivamente sobre el paciente en tanto que reduce la incertidumbre y ansiedad de pacientes y familiares durante el proceso diagnóstico, limita el número de pruebas e intervenciones innecesarias, permite un inicio precoz del tratamiento con riluzol (cuando éste es probablemente más eficaz), favorece una mejor planificación futura y facilita la inclusión en ensayos clínicos en fases más iniciales de la enfermedad2.

Hasta el momento, en España no hay trabajos que analicen el trayecto diagnóstico de los pacientes de ELA. El objetivo de este estudio es por tanto describir el trayecto y retraso diagnóstico de los pacientes con ELA en nuestro entorno y compararlo con lo publicado en otros países.

Pacientes y métodos

Pacientes

Para este estudio se incluyeron pacientes atendidos de forma consecutiva en la Unidad de ELA del Hospital La Fe y diagnosticados de ELA entre octubre de 2013 y julio de 2017.

El Hospital La Fe, en calidad de centro terciario, recibe pacientes remitidos de otros centros públicos y privados para orientación diagnóstica y segunda opinión, o bien pacientes correctamente diagnosticados que desean ser atendidos en la Unidad multidisciplinar de ELA. Por ello, entre los pacientes incluidos en el estudio, algunos fueron diagnosticados en el Hospital la Fe y otros en centros diferentes.

Para los fines de este estudio se seleccionaron únicamente los pacientes con diagnóstico clínico de ELA. Aquellos pacientes que no cumplían criterios de El Escorial en una primera valoración fueron seguidos durante al menos 6 meses, excluyendo aquellos en los que no pudo confirmarse el diagnóstico tras este seguimiento. Tampoco se incluyeron en el estudio los pacientes en seguimiento por neurología por deterioro cognitivo previo al inicio de síntomas motores, así como aquellos diagnosticados de Atrofia Muscular Progresiva (AMP) y Esclerosis Lateral Primaria (ELP), definidas como la afectación exclusiva, durante al menos 4 años, de motoneurona inferior y superior respectivamente17. Finalmente, se excluyeron los pacientes en los que no se disponía de datos suficientes del proceso diagnóstico.

Variables estudiadas

Se trata de un estudio transversal descriptivo a partir de datos recogidos de forma prospectiva y retrospectiva. En la primera visita realizada en el Hospital La Fe, JFVC recogió de forma prospectiva datos demográficos y clínicos: edad, sexo, hospital de procedencia, fecha y región de inicio de síntomas, tasa de progresión18 y categoría de Awaji al diagnóstico16. JFVC, MMM y MFP participaron en la recogida retrospectiva de variables referentes al trayecto diagnóstico mediante la revisión de las notas médicas derivadas de cada atención, registradas en el sistema de información clínico-asistencial hospitalario (programa Orion Clinic) y el Sistema de Información de la Asistencia Ambulatoria de la Agencia Valenciana de Salud (SIA-Gaia), así como de los informes de otros centros no integrados en dichos sistemas, aportados por los propios pacientes. Las variables recogidas incluían: fecha y médico de primera consulta, especialistas visitados y fechas, pruebas realizadas (clasificadas en indicadas o innecesarias, basándonos en las guías europeas de diagnóstico2), fechas de estudios electrofisiológicos (EEF), consultas en urgencias, diagnóstico emitido por el neurólogo, fecha de diagnóstico, fecha de inicio de riluzol.

En las Tablas suplementarias 1 y 2 se detalla cada una de las variables recogidas prospectiva y retrospectivamente. Las dudas surgidas en el proceso de recogida retrospectiva de datos, se resolvieron por consenso de los tres investigadores. En aquellos pacientes que refirieron consultar en los primeros 15 días tras el inicio de síntomas, se revisó con minuciosidad la historia clínica antigua para recoger los síntomas que motivaban una consulta tan rápida. Finalmente, JFVC revisó todos los datos recogidos retrospectivamente.

Análisis estadístico

El análisis estadístico se realizó mediante el programa R y Rstudio versión 3.3.1. Para el análisis descriptivo, se utilizaron medias y medianas (con desviación estándar y rango intercuartil respectivamente) para las variables cuantitativas, y porcentajes para las variables cualitativas. Para el análisis inferencial se utilizó un modelo de regresión lineal en el caso de variables cuantitativas continuas (retraso diagnóstico) y un modelo de regresión logística para variables discretas con distribución Poisson (número de regiones afectas en el EEF según Awaji).

Comité bioética

El estudio contó con la aprobación del Comité Ético de Investigación Clínica del Hospital La Fe. Todos los pacientes firmaron un consentimiento informado para almacenamiento y uso de datos personales.

Resultados

Características de los pacientes

De un total de 186 pacientes valorados y diagnosticados en la Unidad de Enfermedad de Motoneurona en el período comprendido entre octubre de 2013 y julio de 2017, se confirmó el diagnóstico de ELA en 159 pacientes, seleccionando finalmente 143 según los criterios expuestos previamente (Figura 1).

La Tabla 1 resume las características demográficas, clínicas y de trayecto diagnóstico. La edad media fue de 62 años con un predominio de varones y presentación espinal (especialmente en

miembros inferiores [40%], seguido de miembros superiores [26%] y rara vez en musculatura axial [1.4%] o respiratoria [0.7%]). El 8% de los pacientes tenía antecedentes familiares de ELA.

Trayecto diagnóstico global

Los pacientes tardaron en consultar una mediana de 2,5 meses aunque, curiosamente, 20 pacientes (14%) consultaron de forma precoz, en los primeros quince días tras el inicio referido de los síntomas. Las notas médicas no especificaban cómo había sido el debut en 7 de esos pacientes, sin embargo en los otros 13 se describía un inicio agudo expresado en tres formas de presentación diferentes:

Inicio con dolor (8 pacientes): seis de inicio en miembros inferiores y uno de inicio en miembros superiores, que consultaron por dolor lumbar (4), dolor en las extremidades (2) o calambres (2). Un paciente con inicio bulbar consultó por dolor faríngeo.

Inicio tras cirugía (4 pacientes): en este caso refirieron el inicio de los síntomas en el contexto de un postoperatorio (tras intervención traumatológica en tres casos y apendicectomía en otro caso). En tres de ellos el inicio fue en miembros inferiores y bulbar en otro.

Inicio ictal: una paciente debutó con disartria de forma aparentemente brusca, avisando al médico de urgencias quién derivó al hospital ante la sospecha de ictus.

Del total de pacientes, 123 (86%) recibieron asistencia en centros públicos mientras que 20 pacientes (14%) fueron estudiados en centros privados, si bien dos de estos pacientes habían consultado previamente a su médico de familia en la sanidad pública. De los pacientes estudiados en centros públicos, 16 fueron puntualmente visitados también en un centro privado. Por tanto, un 25% del total de pacientes consultaron en un centro privado en algún momento del trayecto diagnóstico.

El retraso diagnóstico medio fue de 13,1 meses (mediana 11.7) desde el inicio de síntomas y el tiempo hasta el inicio de riluzol de 13,3 meses (mediana 12,2). El tiempo invertido en el proceso diagnóstico se puede dividir en tres fases diagnósticas: tiempo que tarda el paciente en consultar, tiempo desde que consulta por primera vez en el sistema sanitario hasta que llega a neurología y tiempo desde que es visitado en neurología hasta que se alcanza el diagnóstico (Figura 2).

Los pacientes tardaron una media de 3,6 meses (mediana 2,5 meses) en consultar con el primer médico (principalmente el médico de familia), quien orientó adecuadamente el caso (derivación a neurología en menos de un mes) en el 38% de las ocasiones.

El tiempo invertido desde que el paciente consulta por primera vez hasta que llega al neurólogo es muy variable, aunque la media es 4,48 meses (mediana 2,8 meses). El neurólogo fue el primer especialista consultado en la mayoría de los pacientes (53%). Pese a ello, un 29% de los casos fueron derivados a otros especialistas tras la valoración por neurología, de manera que hasta el 65% del global de pacientes fue visitado por un especialista distinto al neurólogo en algún momento previo al diagnóstico. Las dos especialidades más consultadas fueron traumatología (26%) y otorrinolaringología (20%). Consecuentemente, en el 50% de los pacientes se realizaron pruebas no informativas para el diagnóstico de ELA (radiografías, endoscopias, etc…) y un 5% de los pacientes fue sometido a una intervención quirúrgica en relación con los síntomas iniciales de la enfermedad (normalmente hernias cervicales o lumbares).

El neurólogo necesitó una media de 5,2 meses y tres contactos para establecer el diagnóstico, con un primer diagnóstico erróneo en un 36% de los pacientes. Todo ello pese a que un 23% de los pacientes ya disponían de EEF en el momento de ser visitados. De modo que gran parte de este retraso ocurre tras haber realizado el primer EEF (Tabla 1). De hecho, en la mitad de los pacientes se necesitó más de un EEF para llegar al diagnóstico, debido a que hasta un 34% de dichos estudios no fueron concluyentes, fundamentalmente (26%) porque no cumplían criterios electrofisiológicos de afectación de ninguna región según los criterios de Awajii. Tampoco fue desdeñable el porcentaje de pacientes (24%) que mostraron una única región afectada en la primera electromiografía. El primer estudio electrofisiológico fue menos informativo (mostró menos regiones de Awaji afectas) cuando el médico solicitante no era el neurólogo (OR = 0,7 [0,50 – 0,96], p = 0,034) (Tabla 2).

Trayecto diagnóstico en centro público respecto a centro privado

La mayor parte de los pacientes atendidos en el sistema público comunicaron los primeros síntomas a su médico de familia, a diferencia de lo observado en pacientes estudiados en centros privados, quienes mayoritariamente consultaron directamente al especialista. El tiempo que

tardaron los pacientes en consultar fue algo superior en el caso de pacientes estudiados en la sanidad privada, posiblemente porque presentaban una velocidad de progresión más lenta. Pese a ello, al tener un acceso más directo a los especialistas, los pacientes visitados en la privada llegaron de media un mes antes al neurólogo. Sin embargo, el retraso diagnóstico fue similar en ambos sistemas, ajustando por edad, forma de inicio y tasa de progresión (p = 0,897; Tabla 3). Esto podría explicarse porque los pacientes estudiados en centros privados visitaron con más frecuencia a especialistas no neurólogos y requirieron en más ocasiones un segundo estudio electrofisiológico para llegar al diagnóstico respecto a los estudiados en la pública, resultando en un mayor tiempo invertido desde que se realiza el primer EMG hasta el diagnóstico (Tabla 1). Por lo demás, el trayecto diagnóstico y el impacto medido en pruebas complementarias o intervenciones no indicadas fueron similares en centros privados y públicos (Tabla 1, Figura 2).

Trayecto diagnóstico según región de inicio

Según el modelo (Tabla 3), los casos espinales presentaron un mayor retraso diagnóstico independientemente de la edad, la tasa de progresión y el sistema en el que fueron estudiados. Esta diferencia puede atribuirse por un lado a un mayor porcentaje de pruebas innecesarias en casos espinales (55%) respecto a bulbares (40%) y, por otro, a mayor porcentaje de pacientes con varios EMG. Además, el tiempo desde el primer EMG hasta el diagnóstico fue mayor en formas espinales respecto a bulbares, pese a que el porcentaje de pacientes que no mostraban afectación de ninguna región neurofisiológica según criterios de Awaji en el primer EMG era similar en los de inicio bulbar (30%) que en los de inicio en miembros inferiores (30%) y superior a los de inicio en miembros superiores (19%).

El mayor retraso en pacientes espinales es atribuible a aquellos de inicio en miembros inferiores. La Tabla 4 muestra que, por un lado, fueron los pacientes peor orientados por el médico de cabecera y por tanto llegaron más tarde al neurólogo. Por otro lado, resultaron los más difíciles de diagnosticar también por los diversos especialistas y el propio neurólogo, lo que se refleja en un mayor número de pruebas innecesarias solicitadas y también más errores diagnósticos. Además, es frecuente que en estos casos no fuera el neurólogo el que solicita el estudio

electrofisiológico, por lo que con más frecuencia requirieron un segundo estudio electrofisiológico y, por ende, se empleó más tiempo y visitas hasta llegar al diagnóstico de ELA.

Por el contrario, las formas de inicio en miembros superiores parecen ser más fáciles de identificar. Como muestra de ello, fueron los pacientes mejor orientados por el médico de cabecera y por tanto fueron derivados con menor frecuencia a especialistas distintos al neurólogo. Además, una vez llegaron a neurología se diagnosticaron más pronto y con menor porcentaje de errores diagnósticos.

Cabe señalar que el perfil de especialistas no neurólogos consultados también varió según la región de inicio, siendo el otorrinolaringólogo el principal especialista consultado en las formas de inicio bulbar y el traumatólogo en los casos de inicio en miembros superiores e inferiores.

Discusión

Casi veinte años después de que se revisaran los criterios de El Escorial, el diagnóstico de la ELA en fases tempranas sigue siendo una asignatura pendiente, como evidencian numerosos estudios realizados en distintos países. A tenor de los resultados de este estudio, nuestro entorno no es una excepción.

Con la aparición de los primeros síntomas, el paciente y sus familiares inician un largo periplo, de unos 12 meses según nuestro estudio y estudios previos (Tabla 5), antes de llegar al diagnóstico. Esta fase pre-diagnóstica característicamente se compone de una serie de etapas en las que el tiempo se reparte más o menos equitativamente, de modo que el retraso diagnóstico no es atribuible al funcionamiento subóptimo de una única etapa sino probablemente a diversos factores en cada una de ellas, que se detallan brevemente a continuación.

Tiempo inicio de síntomas – primera consulta

Los pacientes tardan unos 3 meses en consultar desde que se inician los síntomas, de forma similar a lo descrito en estudios previos (Tabla 5). Sin embargo, un porcentaje no despreciable de los pacientes (14% en nuestro estudio) consultan en menos de 15 días. Si bien esto puede atribuirse en a un recuerdo erróneo de los pacientes de la fecha exacta de inicio de los síntomas, al menos en una parte de estos pacientes se pudo comprobar un inicio agudo en forma de dolor

o tras una cirugía que aparentemente actúa como desencadenante. El inicio de la enfermedad con o precedido por dolor ha sido descrito con anterioridad hasta en un tercio de los pacientes de ELA19 y es un aspecto reseñable porque, según nuestro estudio, estos pacientes parecen consultar de forma precoz. Sin embargo, esta forma de inicio no necesariamente se asocia a un menor retraso diagnóstico, probablemente porque el dolor se considera un síntoma atípico de ELA y sugiere otros diagnósticos. Respecto a la cirugía como desencadenante de los síntomas, un estudio20 mostró que el 3,5% de los pacientes de ELA habían sido operados en los tres meses anteriores al debut de la enfermedad, existiendo una relación entre la región de la cirugía y la de inicio de síntomas, así como una aparente aceleración de la enfermedad tras la cirugía, lo que reforzaría un papel patogénico de la misma.

Tiempo primera consulta - neurólogo

Este tiempo, de unos 4 meses en todos los estudios (Tabla 5), no parece atribuible a la lista de espera si no, en gran medida, a una incorrecta orientación diagnóstica por el médico de cabecera. Únicamente en el 38% de los casos se sospecha precozmente una enfermedad neurológica y aproximadamente la mitad de los pacientes son derivados a distintas especialidades (fundamentalmente otorrinolaringología en los de inicio bulbar y traumatología en los espinales) antes de llegar al neurólogo. Estos datos son, pese a todo, mejores a los encontrados en otro estudio europeo 13.

Tiempo neurólogo – diagnóstico

Este tiempo es quizás el más variable de un paciente a otro (media 5,2 meses, mediana 2,6). En cualquier caso, se antoja excesivamente largo, especialmente si consideramos que los pacientes llevan ya un trayecto de casi 8 meses cuando llegan al neurólogo. Efectivamente, el primer diagnóstico emitido por el neurólogo en la primera visita fue incorrecto en más de un tercio de los pacientes (datos similares a estudios previos, Tabla 5) y muchos fueron derivados a otras especialidades, prolongando el periplo diagnóstico. Esta demora tampoco es atribuible a un retraso en la realización del estudio electrofisiológico ya que la mayor parte del retraso ocurre tras dicho estudio. De hecho, en la mayoría de pacientes se necesitaron varios estudios electrofisiológicos para llegar al diagnóstico.

Factores modificadores del retraso diagnóstico

Al igual que la demora y las etapas diagnósticas, los errores diagnósticos se repiten de forma constante en los diversos estudios, independientemente del trasfondo cultural y las características del sistema sanitario (Tabla 5). De hecho, en el presente estudio, no encontramos diferencias en el retraso diagnóstico en aquellos pacientes estudiados en la pública y en la privada, pese a las diferencias existentes entre ambos sistemas, con acceso más directo (sin mediación del médico de cabecera) al especialista y mayor celeridad en la realización de pruebas complementarias en la atención privada. Efectivamente, observamos que los pacientes estudiados en la privada llegan antes al neurólogo y se realizan antes el EMG. Sin embargo, esto no se traduce en un menor retraso diagnóstico ya que se incrementa el tiempo desde la primera visita al neurólogo y la realización del primer EMG hasta el diagnóstico. Esto sugiere por un lado que el retraso diagnóstico no es atribuible a deficiencias del sistema sanitario como las listas de espera y por otro lado, que una consulta precoz con el neurólogo no siempre garantiza un diagnóstico precoz, como han señalado previamente otros autores8,10,13.

El inicio bulbar y una progresión rápida son dos factores claramente establecidos de menor retraso diagnóstico3,6,15,21,22 que se replican en este estudio. La progresión rápida no sólo condiciona una consulta precoz sino que facilita el diagnóstico de ELA puesto que éste requiere una progresión continua objetivable. Mención especial merece el inicio bulbar. Si bien algunos autores han interpretado que el menor retraso en los pacientes de inicio bulbar es debido a su progresión más rápida15, nuestro estudio demuestra que este efecto es independiente de la velocidad de progresión y que es atribuible, en gran medida, al mayor retraso en las formas de inicio en miembros inferiores. En estos pacientes aumenta la probabilidad de incurrir en errores diagnósticos y realizar pruebas innecesarias ya que, para ojos inexpertos, la ELA puede simular un amplio abanico de patologías altamente prevalentes, especialmente del área traumatológica (hernias discales, patología osteodegenerativa…), con mucha más frecuencia que en las formas de inicio en brazos o bulbares.

Impacto del retraso diagnóstico en el paciente y el sistema sanitario

Los datos de nuestro estudio replican resultados previos y muestran un importante impacto negativo de los errores diagnósticos no sólo sobre el paciente (y sus familiares)7,13 sino también sobre el sistema sanitario, con un gasto cuantificado en unos 3.500 euros por paciente13, 2.000 de los cuales se podrían ahorrar si el diagnóstico se realizara conforme a las recomendaciones de las guías actuales2.

Cómo reducir la demora diagnóstica

Teniendo en cuenta que los errores diagnósticos están condicionados en gran medida por el inicio insidioso y la gran heterogeneidad clínica de la enfermedad, una posible línea de actuación sería la formación específica de aquellos especialistas (médicos de familia, traumatólogos, otorrinolaringólogos…) implicados en el trayecto diagnóstico del paciente de ELA, con especial hincapié en las llamadas banderas rojas. Sin embargo, considerando que un médico de cabecera verá únicamente un par de casos de ELA a lo largo de su carrera profesional, es poco probable que esta medida tenga un efecto significativo en la demora diagnóstica como ya han mostrado estudios previos13, más aún si tenemos presente que llegar antes al neurólogo no asegura un menor retraso diagnóstico8,10,13.

El retraso diagnóstico tampoco parece depender de listas de espera prolongadas ya que, ante la sospecha de ELA, es frecuente que se acelere la realización de estudios (incluso con ingresos programados en muchos centros). Además, intentos previos de crear sistemas de derivación rápida no han conseguido disminuir significativamente el retraso diagnóstico11.

Por tanto, la etapa del trayecto donde parece más razonable incidir para disminuir el retraso diagnóstico, sería desde que el paciente es valorado por primera vez por el neurólogo.

Es evidente que los criterios de El Escorial y sus revisiones posteriores han fracasado en su empeño por propiciar un diagnóstico más precoz. Por ello, y en ausencia de marcadores específicos, el diagnóstico de la ELA sigue siendo clínico, dependiendo fundamentalmente de la experiencia del neurólogo. El EEF representa una herramienta de apoyo de gran utilidad, siempre y cuando se solicite de forma dirigida en base a una sospecha clínica y se interprete de forma adecuada. De no ser así, los estudios pueden resultar subóptimos y, por tanto, no concluyentes, induciendo a confusión, errores diagnósticos y necesidad de repetir la prueba. Cabe resaltar que

algo más de un tercio de los estudios electrofisiológicos realizados por primera vez no fueron compatibles con el diagnóstico de enfermedad de motoneurona según los criterios de Awaji16, fundamentalmente porque no cumplían criterios de afectación de ninguna región. Estos datos son compatibles con estudios previos23 y ponen de manifiesto que los criterios electrofisiológicos de El Escorial y su posterior revisión de Awaji16,24, diseñados para su uso en investigación, no se deben usar en la práctica clínica, especialmente si perseguimos un diagnóstico precoz. No se debe olvidar que determinados fenotipos como la parálisis bulbar y pseudobulbar progresiva muestran característicamente electromiogramas normales en los primeros meses o años de enfermedad y que hasta un 20% de los pacientes de ELA pueden presentar caídas en los potenciales sensitivos en los EEF25. Por ello, un electromiograma normal o con datos atípicos no es incompatible con un diagnóstico de enfermedad de motoneurona cuando la sospecha clínica está bien fundada. En este sentido, la formación específica de neurólogos generales en el diagnóstico de enfermedades de motoneurona y/o la derivación a centros de referencia de aquellos pacientes en que la sospecha diagnóstica no puede ser confirmada podría jugar un papel importante en la reducción de la demora diagnóstica.

Conclusiones

Los datos de este estudio muestran un trayecto y retraso diagnóstico similar al de países de nuestro entorno, que parece ser independiente de las particularidades de cada sistema sanitario e intrínseco a la enfermedad e impacta negativamente sobre el paciente y el sistema sanitario. Las formas de inicio en miembros inferiores constituyen el mayor reto diagnóstico por el amplio abanico de diagnósticos diferenciales. Aunque en cada una de las etapas del trayecto diagnóstico existe margen de mejora, los errores diagnósticos del neurólogo son frecuentes y en parte atribuibles a una mala orientación o interpretación del estudio electrofisiológico. Por tanto, la formación específica del neurólogo y neurofisiólogo general y la derivación precoz a centros de referencia son dos medidas que podrían ayudar a reducir la demora. Se necesitan estudios que analicen si la derivación precoz a centros de referencia permite disminuir la demora diagnóstica.

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Tabla 1. Características de los pacientes y trayecto diagnóstico.

Características		***Global n=143***	***Asistencia pública n=123***	***Asistencia privada n=20***
Edad media al inicio de los síntomas		61,7 (11,97)	61,97 (12,43)	60,04 (8,68)
Sexo (hombre), n (%)		82 (57,34 %)	67 (54,47%)	15 (75%)
Presentación bulbar, n (%)		46 (32,17%)	40 (32,52%)	6 (30%)
Tasa de progresión, mediana (RIQ)		0,78 (0,46, 1,2)	0,8 (0,42, 1,24)	0,69 (0,52, 1)
Médico primera consulta	*Médico de Familia*	105 (73,94%)	103 (83,74%)	2 (10,53%)
	Neurólogo	11 (7,75%)	4 (3,25%)	7 (36,84%)
	Otros especialistas	26 (18,18%)	16 (13,02%)	10 (52,63%)
Pacientes visitados por especialista no neurólogo, n (%)		89 (64,96%)	76 (63,87%)	13 (72,22%)
Nº visitas a Urgencias, mediana (RIQ)		0 (0,1)	1 (0,1)	0 (0,0,5)
Nº visitas a Neurología, mediana (RIQ)		2 (2,4)	2 (2,4)	2,5 (1,75, 3,25)
*Pruebas innecesarias (%)**		50%	51,22%	42,11%
Intervenciones quirúrgicas por los síntomas		6 (4,2%)	5 (4,07%)	1 (5%)
Grado de Awaji	*No cumple criterios*	39 (27,46%)	32 (26,02%)	7 (36,84%)
	Posible	50 (35,21%)	46 (37,4%)	4 (21,05%)
	Probable	37 (26,06%)	31 (25,2%)	6 (31,58%)
	Definido	16 (11,27%)	14 (11,38%)	2 (10,53%)
Diagnóstico definitivo	*ELA*	111 (77,62%)	95 (77,24%)	16 (80%)
	ELA-MNI	28 (19,58%)	24 (19,51%)	4 (20%)
	ELA-MNS	4 (2,8%)	4 (3,25%)	0(0%)
Diagnóstico erróneo inicial emitido por neurólogo (%)		35,71%	35,25%	38,89%
Tiempo inicio síntomas – neurólogo (meses) *Media (DE)* *Mediana (RIQ)*		7,88 (5,76) 6,53 (3,65, 11,58)	8,01 (5,96) 6,6 (3,68, 11,6)	6,91 (4,12) 6,03 (3,53, 9,6)
Tiempo primer EMG – diagnóstico (meses) *Media (DE)* *Mediana (RIQ)*		4,16 (6,17) 1,6 (0,29, 5,57)	3,96 (6,08) 1,33 (0,27, 5,57)	5,4 (6,79) 3,17 (1,67, 5,43)
Tiempo neurólogo – diagnóstico (meses)		5,17 (6,78) 2,65 (0,92, 6,9)	5,14 (6,89) 2,4 (0,9,7)	5,34 (6,14) 3,93 (1,03, 6,83)

Media (DE) *Mediana (RIQ)*			
Retraso diagnóstico (meses) *Media (DE)* *Mediana (RIQ)*	13,07 (9) 11,77 (6,75, 15,8)	13,17 (9,3) 11,9 (7,05, 15,58)	12,47 (7,06) 10,23 (6,71, 17,88)

*****Se consideran pruebas innecesarias, todas aquellas pruebas realizadas durante el estudio de la enfermedad que no figuran entre las indicadas para el diagnóstico de ELA según las guía europeas[2] (pej. radiografía de la mano) o aquellas realizadas de forma reiterativa, innecesariamente. Esta categorización se realizó por consenso en cada paciente.

Tabla 2. Número de regiones afectas según los criterios de Awaji, dependiendo de si el solicitante era o no el neurólogo.

	Neurólogo n = 102, n (%)	No neurólogo n = 38, n (%)
Regiones		
0	22 (21,57%)	14 (38,89%)
1	23 (22,55%)	9 (25%)
2	24 (23,53%)	7 (19,44%)
3	25 (24,51%)	3 (8,33%)
4	8 (7,84%)	3 (8,33%)

Tabla 3. Modelo que estudia los factores que se asocian a un mayor retraso diagnóstico

	Estimado	Intervalo confianza (5% - 95%)	p
Hospital público	0,016	-0,23 – 0,263	0,897
Edad	0,007	0 – 0,015	0,065
Inicio espinal	0,257	0,068 – 0,447	0,008
Tasa de progresión	-0,511	-0,626 – -0,396	<0,001

Tabla 4. Trayecto diagnóstico según región de inicio

	Bulbar n=46	***Miembros superiores n=37***	***Miembros inferiores n=57***
Edad de inicio (años), media (DE)	64,94 (10,86)	59,87 (13,82)	60,67 (11,38)
Tasa de progresión, mediana (RIC)	0,66 (0,37, 1,19)	0,83 (0,53, 1,08)	0,78 (0,54, 1,25)
Buena orientación del médico de familia, n (%)	13 (38,34%)	15 (50%)	13 (31,7%)
Primer especialista, n (%) Neurólogo Otros (total)	 22 (47,83%) 24 (52,17%)	 25 (67,57%) 12 (32,43%)	 26 (47,27%) 29 (52,73%)
Pacientes visitados por especialista no neurólogo, n (%) Traumatología Otorrinolaringología Reumatología Rehabilitación Neurocirugía	33 (76,74%) 2 (4,44%) 27 (60%) 0 (0%) 2 (4,44%) 0 (0%)	19 (52,78%) 10 (27,03%) 1 (2,7%) 3 (8,11%) 5 (13,51%) 4 (10,81%)	36 (65,45%) 23 (41,07%) 0 (0%) 7 (12,5%) 13 (23,21%) 5 (8,93%)
Nº especialista por paciente *Media (DE)* *Mediana (RIC)*	 0,82 (0,53) 1 (1, 1)	 0,8 (0,93) 1 (0, 1)	 1,02 (0,76) 1 (0, 2)
Nº visitas a Neurología, mediana (RIC)	2 (1,75, 3,25)	2 (1, 3)	3 (2, 5)
Pruebas innecesarias, n (%)	18 (40%)	18 (48,65%)	34 (59,65%)
Intervenciones quirúrgicas, n (%)	2 (4,35%)	1 (2,7%)	3 (5,26%)
Médico que solicita EMG, n (%) Neurólogo Otros	 46 (100%) 0 (0%)	 24 (64,86%) 13 (35,14%)	 29 (53,7%) 25 (46,3%)
Primer EMG no concluyente, n (%)	16 (34,78%)	11 (29,73%)	21 (37,5%)
Pacientes con varios EMG, n (%)	15 (32,61%)	19 (51,35%)	37 (64,91%)
Categoría de Awaji al diagnóstico, n (%) Sospecha Posible Probable	 13 (28,26%) 14 (30,43%) 8 (17,39%) 11 (23,91%)	 16 (43,24%) 5 (13,51%) 14 (37,84%) 2 (5,41%)	 8 (14,29%) 30 (53,57%) 15 (26,79%) 3 (5,36%)

Definido			
Diagnóstico erróneo emitido por neurólogo, n (%)	17(36,96%)	10 (27,03%)	22 (40,74%)
Tiempo inicio – neurólogo (meses) *Media (DE)* *Mediana (RIC)*	 7,15 (5,72) 5,22 (3,01, 10,5)	7,66 (5,79) 6,63 (3,97, 11,27)	8,62 (5,92) 7,02 (4,14, 11,75)
Tiempo 1er EMG – diagnóstico (meses) *Media (DE)* *Mediana (RIC)*	 2,74 (4,76) 0,73 (0,17,3,17)	 3,08 (3,8) 1,63 (0,3,3,93)	 6,25 (7,89) 3,97 (0,68, 8,28)
Tiempo neurólogo - diagnóstico (meses) *Media (DE)* *Mediana (RIC)*	 5,52 (7,62) 3,35 (1,14, 6,19)	 4,07 (4,71) 2,1 (0,73, 5,9)	 5,88 (7,32) 4,28 (1,18, 7,56)
Retraso diagnóstico (meses) *Media (DE)* *Mediana (RIC)*	12,7 (9,67) 10,45 (6,54, 16,64)	11,73 (8,35) 11,47 (7,17, 13,4)	14,47 (8,99) 12,9 (8,33, 19,63)

Tabla 5. Retraso diagnóstico de la ELA en estudios previos.

Autor Año	***País***	***Población (n)***	***Errores diagnósticos (%)***	***Tiempos (mediana)***		
				Tiempo a primer médico en meses (mediana)	***Tiempo a neurólogo en meses (mediana)***	***Retraso diagnóstico en meses (mediana)***
Chiò, A. 1999 [4]	Italia España Alemania Argentina Brasil E.E.U.U	201 pacientes Muestra de pacientes de asociaciones de ELA de 6 países	26%-42%	5,6	6 (media)	14
Household am, E. 2000 [8]	Inglaterra y Gales	57 pacientes Muestra de pacientes de una asociación de enfermedades de motoneurona	61%	3	5	16,2
Iwasaki , Y. 2001 [6]	Japón	50 pacientes Muestra de pacientes de centro terciario	----------	5,8	9,7	11,6
Zocolella, S. 2006 [3]	Italia	130 pacientes Muestra poblacional	----------	----------	----------	9,3
Khishchenko, N. 2008 [12]	E.E.U.U	1359 pacientes Muestra poblacional (registro nacional de veteranos)	----------	----------	----------	11

Kraemer, M. 2009 [19]	Alemania	100 pacientes	44%	----------	----------	13,7 *(media)*
Mitchell, J. 2010 [11]	Inglaterra	640 pacientes Muestra de pacientes de centro terciario	----------	----------	----------	12
Cellura, E. 2011 [10]	Italia	260 pacientes Muestra de pacientes basada en un centro terciario	31,1%	3	6	11
Pagano ni, S. 2014 [7]	EEUU	304 pacientes Muestra de pacientes de centro terciario	52%	4	7	11,5
Nzwalo, H. 2014 [20]	Portugal	101 pacientes Muestra de pacientes de centro multidisciplinar	45%	2	6	9,5
Galvin, M. 2017 [13]	Irlanda	155 pacientes Muestra de pacientes basada en un centro terciario	----------	3	8	11

Tabla suplementaria 1 Datos demográficos y clínicos recogidos prospectivamente.

Variable de interés	***Datos recogidos***
Edad de inicio de los síntomas	Considerando como síntomas de inicio: disartria o disfagia; disnea u ortopnea; y debilidad, torpeza o atrofia en algún grupo muscular.
Sexo	Sexo
Hospital de procedencia	Se separó a los pacientes en dos categorías: aquellos estudiados y diagnosticados en el sistema sanitario público (independientemente de que puntualmente realizaran una visita en centros privados) y aquellos en los que el estudio y diagnóstico se realizó en centros privados
Fecha de inicio de los síntomas	Se interrogó al paciente sobre la fecha de inicio de los síntomas y posteriormente se corroboró dicha fecha en el primer registro de notas médicas disponible. En caso de discordancia, se escogió la fecha más plausible (por ejemplo, en caso de que la fecha referida por el paciente fuera posterior a la primera visita médica registrada por dichos síntomas, se escogió lo recogido en la visita médica) o se volvió a interrogar al paciente con la información recogida en la visita médica.
Forma de inicio	Bulbar o espinal
Tasa de progresión	Calculada como: 48-ALSFRS-R al diagnóstico/nº meses desde el inicio de los síntomas[27]
Categoría de Awaji al diagnóstico	No cumple criterios, Posible, Probable, Definida

ALSFRS-R: Escala Funcional para ELA revisada

Tabla suplementaria 2 Datos del trayecto diagnóstico, recogidos retrospectivamente.

Variable de interés	*Datos recogidos*
Primera consulta	Fecha y especialidad del primer médico consultado
Orientación clínica adecuada	Se consideró adecuada cuando el primer médico derivó al paciente al neurólogo en menos de un mes desde la primera visita.
Número de especialistas no neurólogos	Número de especialistas visitados relacionados con los síntomas de ELA.
Consultas en Urgencias	Número de visitas a urgencias por síntomas de ELA antes del diagnóstico
Consulta en Centro Privado	Visita en un centro privado durante el trayecto diagnóstico por síntomas relacionados con la ELA
Consulta en Neurología	Fecha de la primera visita y número de visitas hasta el diagnóstico
Diagnóstico erróneo emitido por el neurólogo	Diagnóstico o sospecha diagnóstica del primer neurólogo en visitar al paciente
Primer estudio neurofisiológico	Médico que solicita el primer estudio neurofisiológico, fecha de realización y resultado. Se consideraron como no concluyentes aquellos estudios que no cumplían criterios electrofisiológicos de afectación de ninguna región o en los que los estudios de conducción nerviosa no permitían descartar otros trastornos como causa de los síntomas, según los criterios de Awaji[28]
Último estudio neurofisiológico	En el caso de pacientes con varios estudios neurofisiológicos, se recogió la fecha del último estudio previo al diagnóstico
Fecha de diagnóstico	Se interrogó al paciente sobre la fecha en que se le comunicó el diagnóstico y posteriormente se corroboró dicha fecha en las notas médicas disponibles. En caso de discordancia, se consideró la fecha de prescripción de riluzol.
Fecha de inicio de riluzol	Fecha de prescripción
Pruebas no indicadas/innecesarias	Todas aquellas pruebas realizadas durante el estudio de la enfermedad que no figuran entre las indicadas para el diagnóstico de ELA según las guía europeas[2] (pej. radiografía de la mano) o en aquellas realizadas de forma reiterativa, innecesariamente. Esta categorización se realizó por consenso en cada paciente.

Nota: la fecha de diagnóstico y de inicio de riluzol se recogieron prospectivamente pero se corroboraron retrospectivamente en las notas médicas disponibles.

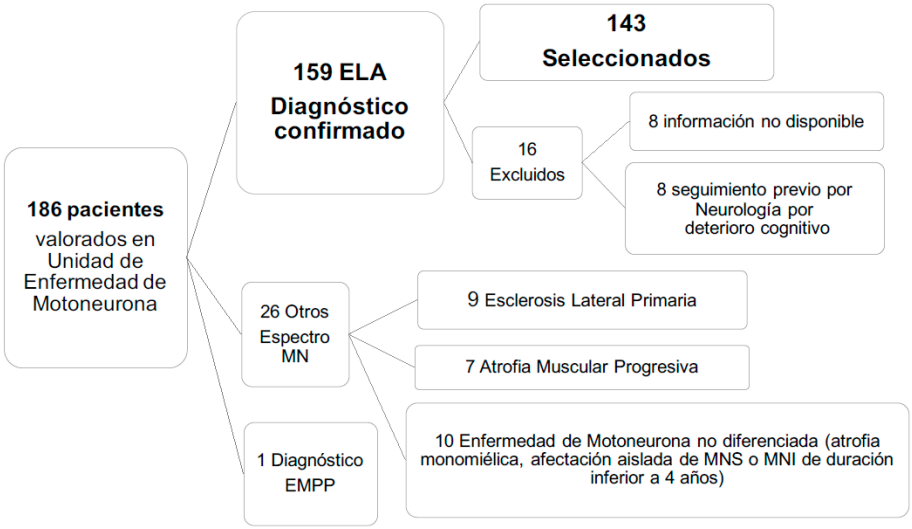

Figura 1. Selección de pacientes

De un total de 186 pacientes valorados en la Unidad de Enfermedad de Motoneurona se confirmó el diagnóstico clínico de ELA en 159 tras un seguimiento de 6 meses. Se excluyeron 27 pacientes diagnosticados de otras entidades que se especifican en la figura. De los 159 pacientes con diagnóstico de ELA, finalmente se seleccionaron 143, tras excluir 16 pacientes por diversos motivos.

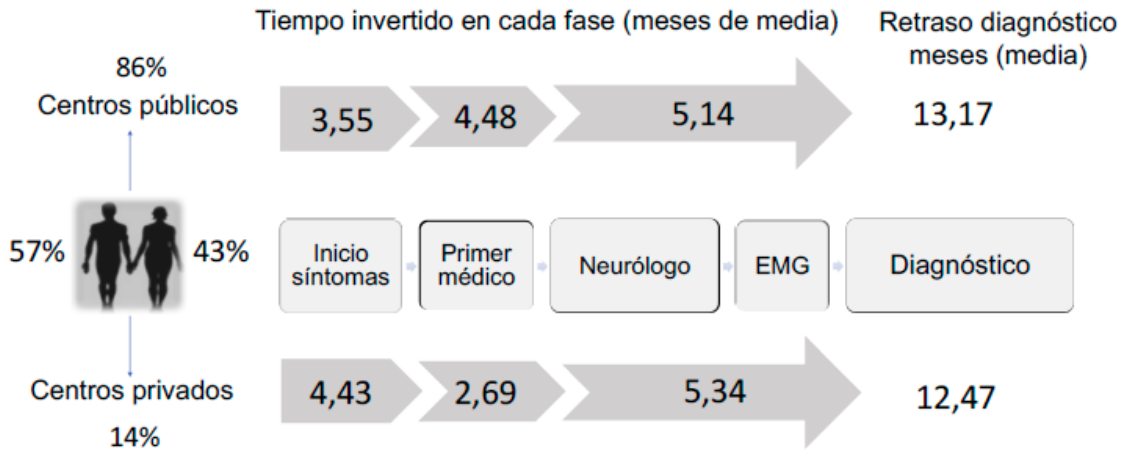

Figura 2. Trayecto y retraso diagnóstico: sistema público vs privado

El periplo que siguieron los pacientes durante la fase pre-diagnóstica de la enfermedad fue similar en ambos sistemas sanitarios, aunque los pacientes atendidos en centros privados consultaron más tarde y llegaron antes al neurólogo por las particularidades de este sistema. Sin embargo, el retraso diagnóstico final fue similar en ambos sistemas sanitarios, ya que el tiempo invertido por el neurólogo en la privada fue algo superior.

G. VÁZQUEZ-COSTA JF, CARRATALÀ-BOSCÀ S, TEMBL JI, FORNÉS-FERRER V, PÉREZ-TUR J, MARTÍ-BONMATÍ L, SEVILLA T. THE WIDTH OF THE THIRD VENTRICLE ASSOCIATES WITH COGNITION AND BEHAVIOUR IN MOTOR NEURON DISEASE. ACTA NEUROL SCAND. 2018; *IN PRESS*.

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The width of the third ventricle associates with cognition and behaviour in motor neuron disease

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Running Title: Third ventricle: a marker of cognitive impairment in MND

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ABSTRACT

Objectives

An enlarged width of the third ventricle (WTV) has been described in amyotrophic lateral sclerosis (ALS) patients, although its clinical meaning is unknown. The aims of this study were to evaluate the contribution of demographical, clinical and genetic factors to the WTV in different motor neuron disease (MND) phenotypes and to assess its brain structural correlates.

Materials & methods

The WTV was measured by transcranial ultrasound in 107 MND patients (82 diagnosed with classical ALS, 16 with progressive muscular atrophy and 9 with primary lateral sclerosis) and 25 controls. Genetic analysis, and neurological and neuropsychological examinations were performed in patients. Brain volumetric analysis of MR images were obtained in 85 patients. The association of WTV with demographic, clinical, genetic and neuropsychological variables as well as with brain volumes was assessed by multivariable models.

Results

Eighteen patients were diagnosed with genetic MND and 42.3% of patients showed executive or behavioural impairment (EBI). MND patients showed larger WTV than controls. The WTV was significantly associated with age, spinal onset and the presence of EBI, but not with the genetic background, the phenotype, or disability. Greater WTV was also associated with reduced subcortical gray matter volume, but not with the cortical or the white matter volume.

Conclusions

The enlargement of the WTV found in the different MND phenotypes is attributable to the subcortical gray matter atrophy and is associated with cognitive and behavioural impairment. Larger longitudinal studies are needed to determine its role as biomarker in MND patients with frontotemporal dementia.

Keywords: motor neuron disease; amyotrophic lateral sclerosis; progressive muscular atrophy; primary lateral sclerosis; third ventricle; ultrasound; cognitive impairment.

Introduction

Motor neuron diseases (MND) are neurodegenerative conditions involving upper (UMN) and/or lower motor neurons (LMN).[1] Three main phenotypes can be distinguished according to the degree of UMN and LMN impairment:[1] classical amyotrophic lateral sclerosis (cALS), primary lateral sclerosis (PLS), and progressive muscular atrophy (PMA). Beyond motor neurons, MND patients show involvement of other brain structures in both neuroimaging and pathological studies.[2–5] These changes might be related to the presence of extra-motor features, such as cognitive or behavioural impairment in 20% to 50% of MND patients.[1,5–7] Language and dysexecutive deficits are the most frequent cognitive findings, a poor letter fluency being the hallmark of the latter.[6–8]

Transcranial sonography can measure the width of the third ventricle (WTV) in an easy and reliable manner, showing a good correlation and agreement with its manual measurement in magnetic resonance (MR) imaging.[9]

The WTV is believed to indirectly reflect the degree of subcortical brain atrophy, particularly on adjacent structures such as thalamus.[10] In this way, it has been shown useful to monitor progressive brain atrophy associated with aging, as well as for the diagnosis or monitoring of progressive supranuclear palsy (PSP) or multiple sclerosis patients.[11–13] The WTV also correlates inversely with the cognitive performance in both healthy subjects and patients with multiple sclerosis, Alzheimer's disease and Parkinson's disease.[11,14–17] Recently, an enlarged WTV has been

described in cALS patients,[18,19] although its clinical correlate is not known. Moreover, the WTV has been studied neither in other phenotypes (PMA or PLS) nor in familial MND.

The aims of this study were: (1) to assess the differences in the WTV between controls and different MND phenotypes; (2) to evaluate the contribution of demographical, clinical and genetic factors to this biomarker and; (3) to analyse its brain structural correlates.

Methods

Subjects and definitions

Patients diagnosed with cALS, PMA or PLS were recruited and evaluated between February 2014 and November 2017 by the same neurologist (JFVC). The cALS patients met the revised El Escorial criteria of possible, probable or definitive ALS [20]. Patients presenting with a progressive isolated LMN impairment, affecting at least two regions, were diagnosed with PMA after appropriately excluding other LMN syndromes.[21] PLS was defined as a progressive isolated UMN impairment in at least one region other than the lumbar region, lasting more than four years.[22] Patients with stroke history were excluded. The hyperechogenicity of the substantia nigra was also assessed in a subgroup of these population and the results have been published elsewhere.[23] For comparison purposes, a cohort of controls without neurological diseases was also recruited.

Genetic MND

MND patients were systematically asked for family history of MND or dementia. Patients were categorised as familial MND whenever they had a positive family history as previously defined,[24] or as sporadic MND when not. Both sporadic and familial MND patients were screened for *C9ORF72* with repeat primed PCR and expansions were confirmed with Southern blotting. Moreover, familial MND patients not carrying a *C9ORF72* expansion, *SOD1*, *TARDBP* and *FUS* genes were subsequently analysed by Sanger sequencing. Finally, familial MND patients and sporadic MND patients carrying mutations were classified as genetic MND (gMND).

Clinical examination

Age, gender, date of onset of motor symptoms, region of onset (bulbar and spinal), disability (ALSFRS-R), and the degree of UMN impairment (UMN score),[25] were recorded for all MND patients.

Verbal fluency (letter P) was examined in non-anarthric patients and the verbal fluency index[8] was subsequently calculated. Four anarthric patients without disability in the dominant hand were examined with both the reverse digit span and the trail making test. Cut offs of each executive test were defined by the normative values, adjusted by age and education level, published in the Spanish population.[26,27]

Moreover, the diagnosis of behavioural frontotemporal dementia (bvFTD) was based on the Rascovsky criteria,[28] after interviewing the caregiver about the presence in the patient of: a) disinhibition, b) loss of sympathy and empathy, c) perseverative, stereotyped or compulsive

behaviour, d) hyper-orality/dietary change, e) apathy. Mild executive, mild behavioural impairment and bvFTD were diagnosed according to current criteria.[8] These criteria require the presence of either an impaired verbal fluency or two other executive non-overlapping measures for the diagnosis of executive impairment; the identification of either apathy or two other behavioural symptoms for the diagnosis of mild behavioural impairment; and the identification of at least three behavioural Rascovsky criteria for the diagnosis of bvFTD.

A subset of patients were also assessed with the frontal assessment battery (FAB) (n=79), which ranges from 0 to 18, with higher scores indicating better cognition. For the statistical analysis, the item-adjusted FAB scores were calculated as previously published[29] according to the formula: item-adjusted score = original score $*$ 100/% of items performed. Moreover, in some patients (n=61) the Spanish validated version of the Frontal Systems Behaviour Scale (FrSBe),[30] was carried out by the caregiver. The FrSBe is composed of 46 behavioural items, each of them ranging from 1 (almost never) to 5 (almost always), which are grouped in three subscales: apathy, disinhibition, and executive dysfunction. For the present study, we have considered the Z-scores of the post-illness forms.[30]

Transcraneal sonography examination

An expert neurologist sonographer (JIT), blind to the clinical data, performed the ultrasound examination with the same system (Toshiba Aplio XG, Tokyo, Japan 2008), equipped with a 2.5 MHz phased-array transducer to obtain B-mode images through a temporal acoustic bone window. The WTV was obtained and measured by this examiner, as previously published.[31]

MR imaging acquisition and processing

MRI examinations were performed on a 3T MRI scanner (Signa HDxt, GE Healthcare, Milwaukee, USA) using a transmit-receive head coil array with 8 elements. The MR imaging protocol included a 3D T1-weighted axial fast spoiled gradient recalled sequence (TR, 6.6 ms; TE, 2.8 ms; TI, 400; FOV, 220 x 220 mm^2; matrix, 256 x 256 mm^2; flip angle, 12º; voxel size 1 x 0.94 x 0.94 mm^3).

Brain volumes were automatically segmented and measured by FreeSurfer version 5.2 (http:// surfer.nmr.mgh.harvard.edu/). Segmentations were subsequently inspected and errors were corrected when present. For the purpose of this study, besides the third ventricle volume, following global measures of brain volumes provided by FreeSurfer were considered: total intracranial volume (TIV), cortex, subcortical gray matter (which includes thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens and the ventral diencephalon) and white matter.

Statistical analysis

Data were summarized using mean (standard deviation) and median (1^{st} and 3^{rd} quartiles) in the case of continuous variables and relative and absolute frequencies in the case of categorical variables. WTV age- and gender-controlled differences between patients and controls, and between sporadic and genetic MND patients, were assessed with a linear regression model. The association of the demographical and clinical variables of the MND patients with the WTV was analysed with linear regression models. Firstly, a group of clinical covariates (age, gender, phenotype, gMND, disease duration, ALSFRS-R, progression's rate and executive or behavioural

impairment) were pre-selected based on previous literature and the study goal. Finally, the combination of covariates that best fitted the model was selected according to the Akaike Information Criteria (AIC). Since the larger brains (TIVs) associate with larger brain structures (including the third ventricle),[32] we repeated the best clinical model but controlling for TIV in the 85 subjects with available MRI. Moreover, the association of brain volumes with the WTV, were also assessed with a linear regression model. The models were extended with the variable "Patient" as a random effect with random intercept in order to correct the non-independence of the data. Pearson's correlation, with a false discover rate correction to adjust for multiple comparisons, was performed to study the relationship between WTV and cognitive and behavioural tests. Finally, the diagnostic accuracy of the WTV for cognitive impairment was analysed with the bootstrap-validated AUC, after controlling for those variables that associated with the WTV in previous models. P values < 0.05 were considered statistically significant. All the statistical analyses and graphs were carried out using R software (version 3.4.3).

Ethical approval

The study was approved by the Ethics Committee for Biomedical Research of the La Fe Hospital (Valencia, Spain) and has been conducted according to the principles of the Declaration of Helsinki. All the participants, or their next of kin in those incompetent ALS-FTD patients, gave written informed consent.

Results

Population characteristics

The study included 117 MND patients (91 cALS, 16 PMA and 10 PLS) and 25 controls. The demographical, clinical, genetic and ultrasound characteristics of both, patients and controls, are summarised in Table 1. Overall, MND patients were moderately disabled and the mean time from symptoms onset ranged between 14 months in cALS to 55 months in PMA patients. Eighteen patients (15.4%) were classified as gMND. Thirteen patients could not be cognitively examined due to great motor disability. Some degree of executive or behavioural impairment (EBI) was diagnosed in 44 (42.3%) of the remaining patients: 18 patients (17.3%) were diagnosed with isolated mild dysexecutive impairment; 11 patients (10.6%) with mild behavioural impairment; 9 patients (8.6%) showed both mild dysexecutive and behavioural impairment; and 6 patients (5.8%) with FTD.

WTV in patients and controls

Ten MND patients (8%) showed a poor temporal acoustic bone window. Therefore, the WTV could be measured in 107 patients. MND patients showed larger WTV than the healthy controls (Estimate = 0.89 [0.04, 1.76], p=0.042), after controlling for age and sex. Moreover, age (Estimate = 0.09 [0.06, 0.12], p<0.001) and male sex (Estimate = 0.95 [0.27, 1.62], p=0.006) were strongly associated with greater WTV in the whole cohort.

Modifiers of the WTV in the MND patients

Genetic MND was not associated with changes in the WTV after controlling for age and sex (Estimate = 0.93 [-0.151, 0.336]) and the WTV appeared similar independently of the causal mutation (Figure 2).

In the multivariable analysis, age, male sex and EBI, but not other clinical variables (phenotype, site of onset, disease duration and ALSFRS-R), were directly associated with the WTV (Table 2). When controlling for the TIV, the effect of male sex on the WTV disappeared, and the effect of EBI and of spinal onset increased, the latter reaching the statistical significance (Table 3).

WTV and cognitive impairment

The WTV correlated with the adjusted FAB (r = -0.68), the VFI (r = 0.37) and the dysexecutive (r = 0.51) and apathy (r = 0.48) subscales of the FRSBE (Figure 3).

Although MND-bvFTD patients consistently showed larger WTV than age and sex matched cognitive normal patients (Figure 4), there was considerable overlap on the WTV between all three cognitive subgroups (Figure 5). Moreover, the WTV showed limited diagnostic accuracy to predict EBI even when controlled either by age and sex (AUC 0.794) or by age, site of onset and TIV (AUC 0.776).

WTV and brain volumes

MR images were available in 85 patients within 3 months of the sonographic exam. A strong correlation between the WTV and the third ventricle volume (as per MR) was found (r = 0.887

[0.83, 0.93]; Figure 6). In the multivariable analysis, after controlling for the TIV, greater WTV was associated with reduced total subcortical gray volume (Estimate = -0.211 [-0.393, -0.03], p = 0.023), but not with the cortex (Estimate = -0.028 [-0.253, 0.197], p = 0.806) or the white matter volume (Estimate = -0.19 [-0.393, 0.014], p = 0.067).

Discussion

Our study confirms previous reports[18,19] of an enlarged WTV in cALS patients and demonstrates that this enlargement can be also found in other MND such as PMA and PLS, since it is not associated with the motor phenotype, but with the presence of EBI.

In healthy controls, the WTV is known to be enlarged in male subjects, due to their overall larger brain volumes, and to progressively increase with aging.[11] Moreover, as a marker of brain atrophy, it is also enlarged in a great variety of neurodegenerative diseases,[13] particularly in PSP patients,[31] where it has been proposed as a progressions biomarker.[33]

Interestingly, the WTV predicts future cognitive impairment in aged controls.[11] Furthermore, it also correlates with cognitive performance in multiple sclerosis, Alzheimer's or Parkinson's disease.[14–17]

Although the WTV is considered to be a marker of subcortical brain atrophy, particularly the thalamus,[10] neuroimaging studies addressing the association of specific brain regions with the WTV are lacking. Therefore, the anatomic correlates and exact meaning of the WTV enlargement in ALS remain unknown.

The WTV in MND

The WTV has been previously found to be similar in cALS and Parkinson's disease patients[34] but larger than in controls.[18,19] Moreover, the third ventricle's volume is known to increase in cALS with the disease duration.[35] However, the clinical correlates of this structure have been scarcely studied and the results are either controversial,[18,19,34] or showing several limitations such as the lack of control by age, sex and TIV or the use of unsuitable cognitive tests (MMSE).[18]

In our study, the WTV was greater in MND patients than controls. As expected, the WTV was influenced by age but not by sex after controlling for TIV, confirming that males have larger WTV because they have larger brains.[32] However, neither motor phenotypes nor the disease duration or disability were associate with the WTV. Conversely, the WTV was independently associated with the spinal onset, after controlling for TIV. A previous study showed larger WTV in bulbar onset patients,[34] while another study failed to find differences.[19,34] However, these studies did not control for co-variables (such as age, sex or cognitive impairment) that influence the WTV and are unevenly distributed in spinal and bulbar onset patients. Previous MRI studies have shown larger atrophy of motor-related gray and white matter structures in spinal onset patients even after controlling for disability, whereas extra-motor structures were more frequently impaired in bulbar onset patients, probably related with the increased frequency of cognitive impairment.[36,37] Since our model controlled for cognitive impairment, the larger WTV found in spinal onset patients could actually reflect a larger atrophy in motor-related subcortical structures.

The WTV also independently associated with the EBI, an association which increased after controlling for TIV. In MND, there is a continuum of cognitive and behavioural changes, which seems to be independent of the motor phenotype.[6,7] In our study, the WTV correlated with those tests assessing executive tasks and apathy, which are hallmarks of these cognitive and behavioural changes in MND patients.

Moreover, we found that the subcortical gray matter volume, but not the cortex or the white matter volumes, were associated with the WTV. The subcortical gray matter volume comprises basal ganglia, thalamus, hippocampus and accumbens among other structures. Interestingly, atrophy of these structures has been found in ALS-FTD *vs.* cognitively normal ALS patients.[6] Moreover, atrophy in thalamus appears to mirror the progressive frontotemporal cortical involvement in ALS patients.[38] Given their anatomical localization is not surprising that the atrophy of subcortical gray matter structures results in an enlargement of the WTV. Furthermore, recent pathologic and neuroimaging studies have confirmed the presence of widespread brain atrophy and TDP-43 pathology in a subgroup of ALS patients characterized by higher rates of cognitive impairment[3,39] and poor prognosis.[40] Altogether, our study suggests that the WTV enlargement in some MND patients is largely attributable to the atrophy of subcortical gray matter structures involved in executive and behavioural tasks and could therefore be a marker of the extra-motor disease spread.

Despite the correlation of the WTV with cognitive and behavioural tests, the ability to predict EBI based on the WTV was limited, mainly because there was considerable overlap in the WTV between mild cognitively impaired and cognitively normal patients. The diagnostic accuracy of

the WTV for the bvFTD diagnosis could not be analysed due to the low number of bvFTD-MND cases. Moreover, since only executive tests were performed in this study, the impairment in other cognitive areas might have been missed in subjects considered to be cognitive normal, limiting the specificity of the WTV. Furthermore, other non-controlled factors such as vascular risk factors could influence the WTV. A longitudinal assessment of the WTV would account for these interindividual differences, and might allow the prediction or monitoring of a progressive cognitive impairment. As a progression's biomarker would be especially suitable for ALS patients, since it can be easily obtained in advanced disease stages (even in patients requiring ventilation support) at bedside and does not require post-processing, unlike MRI-based biomarkers.

Finally, unlike the hyperechogenicity of the substantia nigra,[23] our study was unable to find differences in sporadic *vs.* genetic MND patients. This result should be considered with caution due to the low number of gMND patients and the heterogeneity of the causal mutations. Indeed, different mutations cause diverse patterns of brain atrophy.[3,41]

Strengths and limitations

Our study represents the largest and most thorough study of the clinical meaning of the WTV in ALS patients. We included phenotypes (PMA and PLS) and variables that have not been analysed before and, by using multivariable analysis, also limit some methodological pitfalls of previous studies. Moreover, the structural correlates of the WTV were also assessed. However, our study also has some limitations. First, a larger and better characterised cohort of controls would have been desirable. However, this cohort was big enough to find statistically significant differences

with the cohort of patients, after correcting for age and sex. Second, the sample size for some studied variables (genetic ALS or bvFTD-ALS) was small as their prevalence is low. Third, although the diagnosis of executive and behavioural impairment followed current diagnostic criteria,[8] a more comprehensive neuropsychological examination could have increased the accuracy of the WTV as a marker of cognitive impairment. Moreover, FrSBe has not been validated in ALS patients and behavioural changes (in particular apathy) could have been overestimated due to motor impairment. However, FrSBE is a widely used questionnaire for the behavioural examination in ALS patients that has been validated in the Spanish population, whereas ALS-specific questionnaires have not. Moreover, in the apathy items, relatives were specifically instructed to rate patients' initiative beyond the motor impairment.

Conclusions

We show that the enlargement of the WTV found in MND patients is attributable to the atrophy of subcortical gray matter structures and associates to cognitive and behavioural impairment. Larger longitudinal studies are needed to determine its role as a diagnostic or prognostic biomarker, especially in ALS-bvFTD patients.

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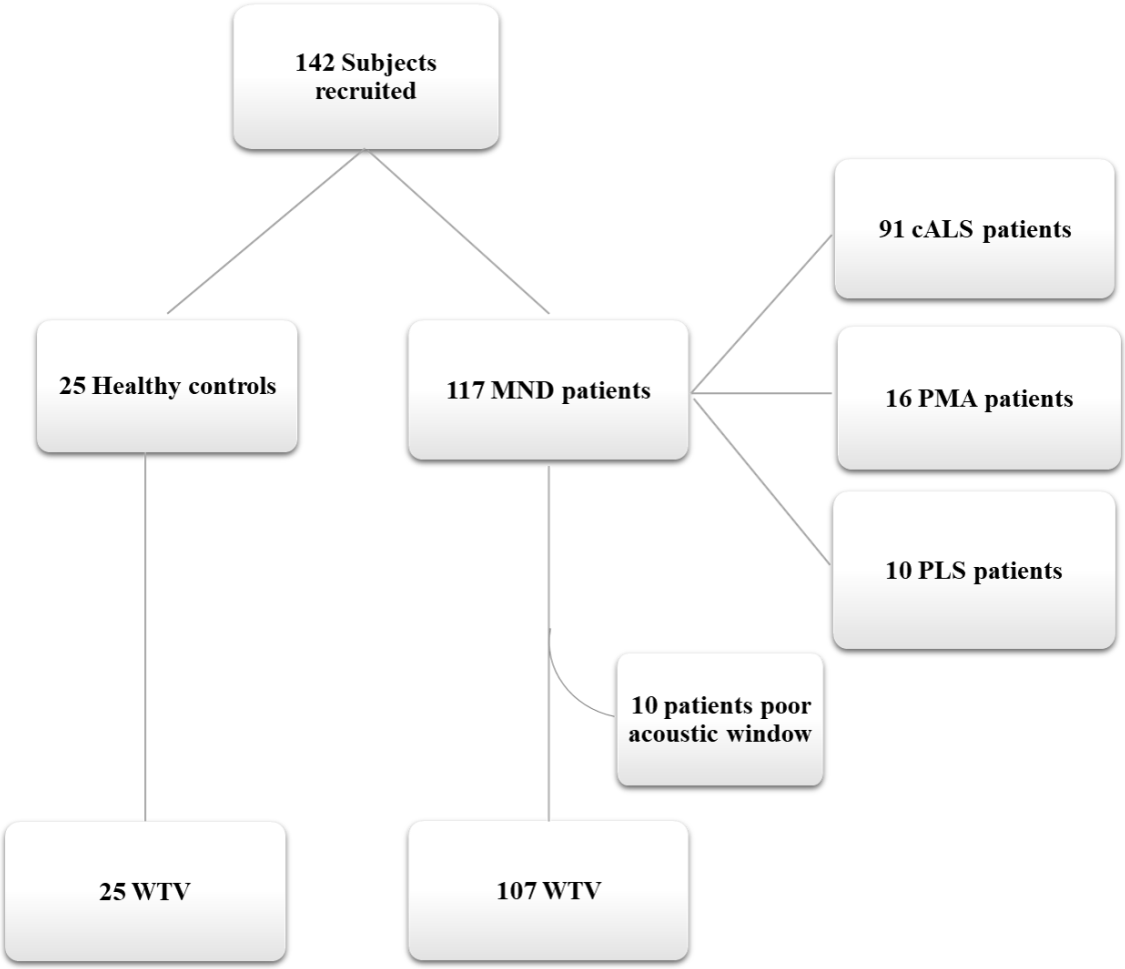

Figure 1. Diagram representing the flow of participants. Ten patients were excluded from the final analysis because of poor acoustic bone window. cALS: classical amyotrophic lateral sclerosis; MND: motor neuron disease; PLS: primary lateral sclerosis; PMA: progressive muscular atrophy; WTV: width of the third ventricle.

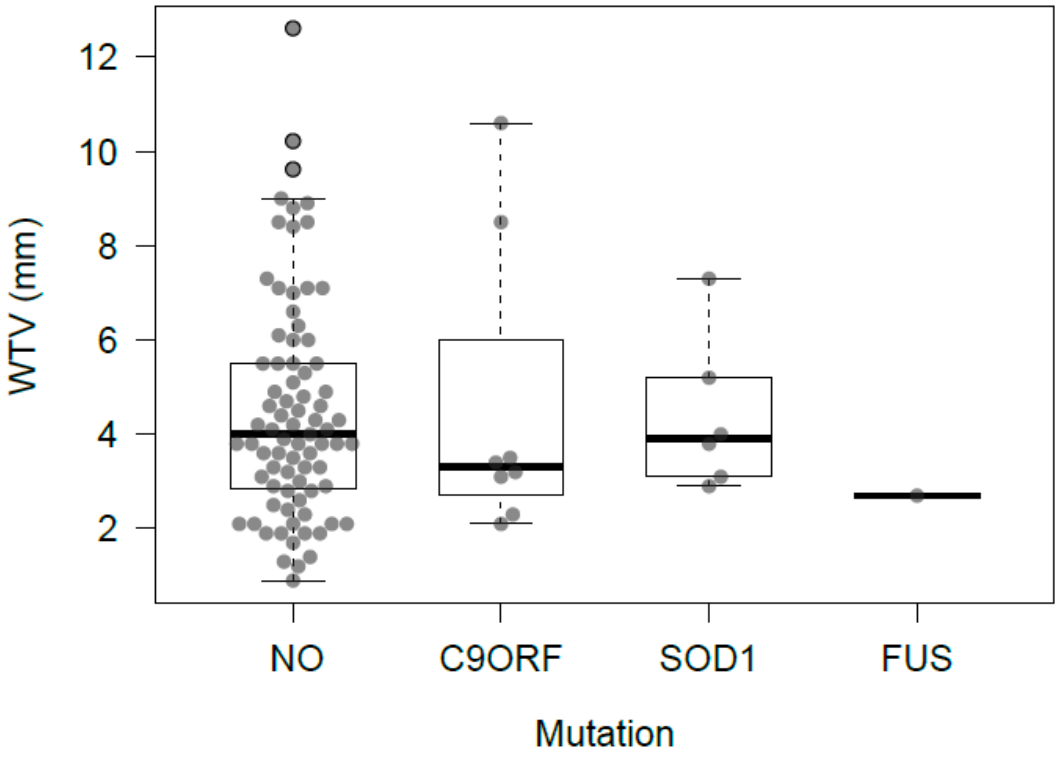

Figure 2. WTV in MND patients harbouring mutations ("C9ORF72", "SOD1" and "FUS"), familial ALS without known mutations ("Unknown") and sporadic patients not carrying mutations ("NO"). WTV: width of the third ventricle.

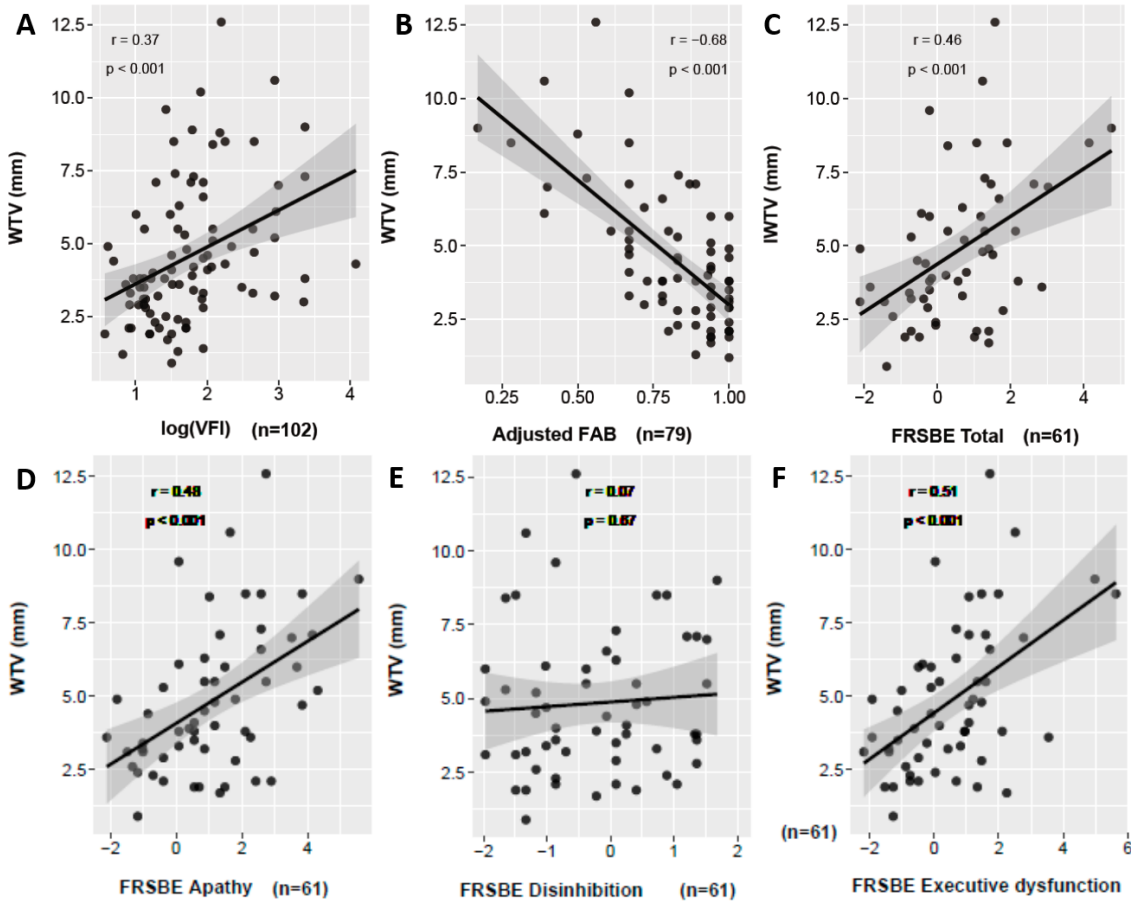

Figure 3. Correlation of the WTV with the results of executive (A and B) and behavioural tests (C-F). FRSBE values correspond to Z-scores. FAB: frontal assessment battery; FRSBE: frontal systems behavioural scale; VFI: verbal fluency index; WTV: width of the third ventricle.

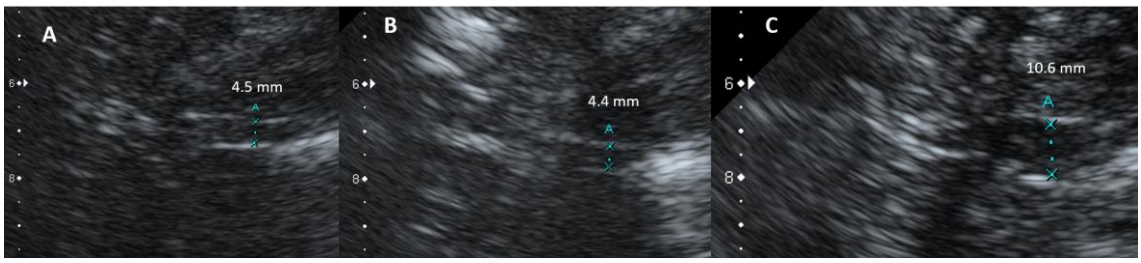

Figure 4. WTV in two cALS patients and one control matched by sex (all male) and age: 67 years old control (A); 65 years old cognitive normal cALS patient (B); 66 years old

cALS-bvFTD patient (C). The WTV is considerably larger in the cALS-bvFTD patient. cALS: classical amyotrophic lateral sclerosis; bvFTD: behavioural frontotemporal dementia; WTV: width of the third ventricle.

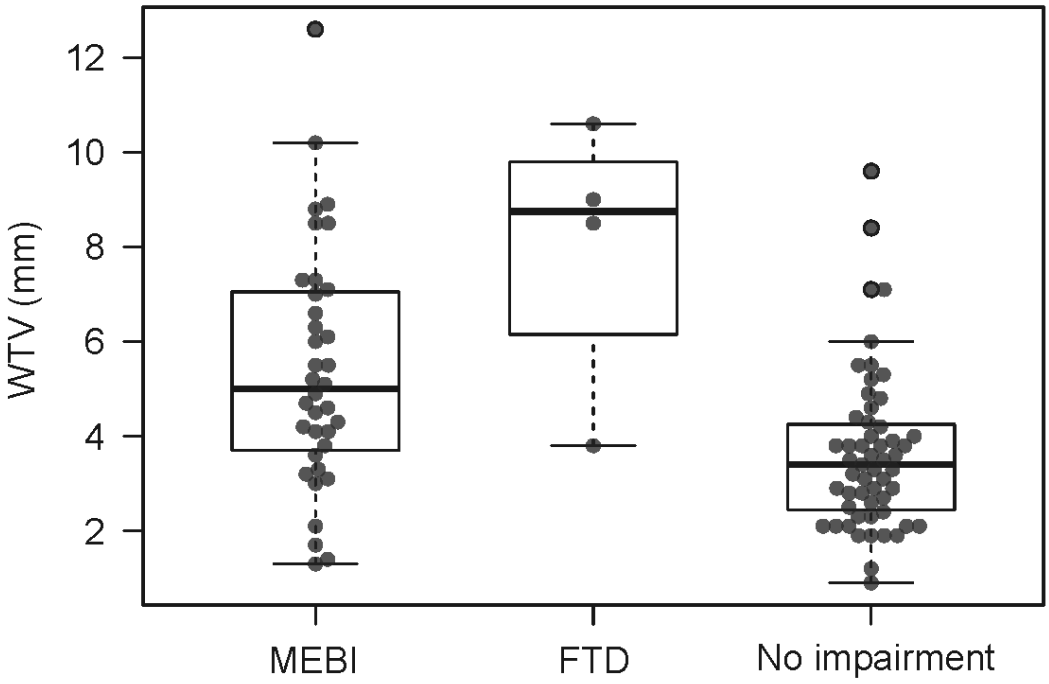

Figure 5. WTV in MND patients according to their cognitive and behavioural classification. MEBI: mild executive or behavioural impairment; WTV: width of the third ventricle.

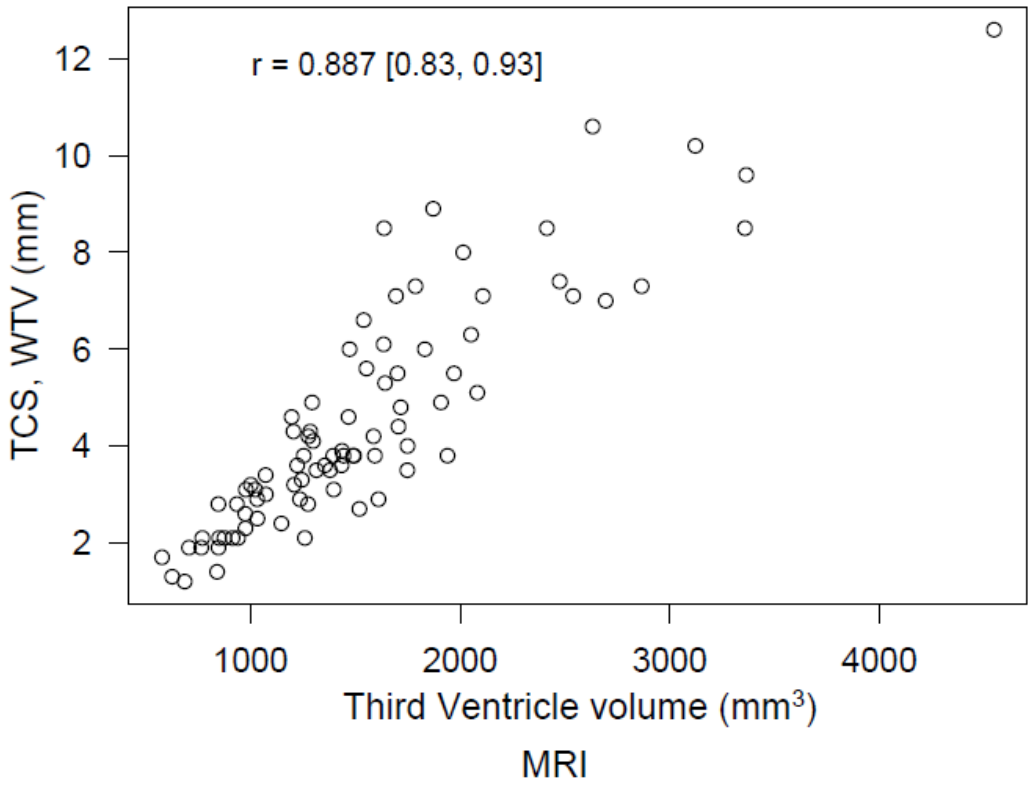

Figure 6. Correlation between the third ventricle volume as per MRI and the WTV as per transcranial sonography. TCS: transcranial sonography; WTV: width of the third ventricle.

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New insights into the pathophysiology of fasciculations in amyotrophic lateral sclerosis: an ultrasound study

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ABSTRACT

Objective

To describe the fasciculation pattern in ALS and to analyse its clinical and pathophysiological significance.

Methods

Ultrasound of 19 muscles was performed in 44 patients with a recent diagnosis (<90 days) of ALS. The number of fasciculations was recorded in each muscle and the muscle thickness and strength were additionally measured in limb muscles. A subgroup of patients were electromyographically assessed.

Results

US was performed in 835 muscles and EMG was available in 263 muscles. US detected fasciculations more frequently than EMG. Fasciculations were widespread, especially in upper limbs onset patients and in the cervical region. Fasciculations' number inversely associated with ALSFR-R and body mass index (BMI) and directly with BMI loss and upper motor neuron (UMN) impairment. Our statistical model suggest that fasciculations increase with the initial lower motor neuron (LMN) degeneration, reach their peak when the muscle became mildly to moderately weak, decreasing afterwards with increasing muscle weakness and atrophy.

Conclusions

Our study suggests that both UMN and LMN degeneration trigger fasciculations causing BMI loss. The degree of LMN impairment could account for differences in fasciculations' rates within and between muscles.

Significance

In ALS, fasciculations could explain the link between hyperexcitability and BMI loss.

Keywords: Amyotrophic lateral sclerosis; fasciculations; ultrasound; hyperexcitability; pathophysiology.

Highlights

- Fasciculations are more frequent in proximal and cervical muscles and in upper limb onset patients

- Fasciculations associate directly with BMI loss and UMN impairment and inversely with disability
- Fasciculations increase with the initial LMN impairment but they decrease when it progresses

Abbreviations

LL: lower limbs; LMN: lower motor neuron; MRC: muscle strength based on the medical research council scale; MTh: muscle thickness; UL: upper limbs; UMN: upper motor neuron; US: ultrasound.

1. INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects the upper (UMN) and lower motor neurons (LMN). Fasciculations are involuntary twitches due to spontaneous contraction of muscle fascicles, originating from motor unit depolarizations as recorded by electromyography (EMG) (de Carvalho et al., 2017). Widespread fasciculations are characteristic of ALS and, therefore, have been incorporated to the diagnostic criteria (de Carvalho et al. 2008).

Ultrasound (US) imaging, which allows the examination of a wide muscle area and several muscle fascicles simultaneously, has been proven more sensitive than EMG for fasciculations' detection in ALS (Walker et al. 1990; Misawa et al. 2011). Moreover, US is a painless technique and can be more suitable than EMG for a widespread examination.

Previous studies have studied the origin of fasciculations based on their electrophysiological characteristics (de Carvalho et al. 2017). However, the clinical factors associated with them have been scarcely studied and consequently its clinical meaning remains largely unknown. Unravelling these associations may lead to a better understanding of their pathophysiology.

The aim of this study is to describe the ultrasonographic pattern of fasciculations in several muscle groups in patients recently diagnosed with ALS and to analyse the relationship between these patterns and several demographic, clinical, analytical and neurophysiological variables.

2. MATERIAL AND METHODS

2.1 Study population

This observational study prospectively enrolled patients with a recent (< 90 days) clinical diagnosis of ALS. Patients were classified into three phenotypes according to the degree

of UMN and LMN impairment at diagnosis: LMN predominant ALS (LMN-ALS) patients showed none or equivocal UMN signs upon recruitment; UMN predominant ALS (UMN-ALS) patients showed no clinical or neurophysiological signs of LMN impairment; and classical ALS patients showed both signs. All patients were diagnosed, recruited and examined by the same experienced neurologist (JFVC) in the ALS Unit of Hospital La Fe. Patients not meeting Awaji criteria at recruitment, were followed up until death or for at least 12 months and the emergence of new LMN and UMN signs was monitored. At the end of the study, the ALS diagnosis was confirmed in all the included patients, based either on Awaji criteria (de Carvalho et al. 2008) or in a compatible clinical course (progressive involvement of several regions including either bulbar or respiratory impairment) in those patients who did not developed UMN signs.

2.2 Clinical and analytical variables

Age, sex, dates of symptoms onset and of diagnosis, site of symptoms onset, disability (ALSFRS-R) (Cedarbaum et al. 1999), and degree of UMN impairment (UMN score) were recorded for all the patients upon recruitment. The UMN score measures the degree of UMN impairment ranging from 0 to 16 (Vázquez-Costa et al. 2018). It was calculated by summing up the number of pathologically brisk reflexes (brisk facial and jaw jerks; biceps, supinator, triceps, Hoffmann, knee and ankle reflexes; and extensor plantar responses). Premorbid body mass index (BMI) was estimated by systematically asking patients about their usual weight the year before symptoms onset. Moreover, medical records were reviewed to confirm this datum, although it was not available for all the patients. Only mild discrepancies between the weight reported by the patient and the one registered in medical records were found (≤ ± 1 kg). In those cases, the latter was chosen for this study. The percentage of BMI loss was calculated as follows: (premorbid BMI - BMI at recruitment)*100/premorbid BMI . The muscle strength, measured with the modified Medical Research Council (MRC) rating scale (ranging from 0 to 5 and including grades 4- and 4+), was assessed in all US examined limb muscles (see below).

Serum creatinine levels at diagnosis were recorded and the results are reported in mg/dL.

2.3 Ultrasound study

An experienced neuroradiologist (JIT), blind to clinical details, performed US examinations at recruitment with a Toshiba Aplio XG (Tokyo, Japan 2008) equipped with a 7.2-14 MHz linear-array transducer. All system-setting parameters, such as global gain (80 dB), time gain compensation (in neutral position), depth (30 mm for cricothyroid and ABP muscles and 50 mm for the remaining muscles), width (38 mm), frequency (13 MHz), the position of the transducer, compression and focus were kept constant throughout the study. Ten muscle groups representing the 4 anatomical regions were examined in the transverse plane in a supine relaxed patient as previously reported (Martínez-Payá et al. 2017a, 2017b, 2018). In the bulbar region we studied the tongue and cricothyroid; in the cervical region, the biceps brachialis, triceps brachialis and the abductor pollicis brevis (APB) muscle ; in the thoracic region the rectus abdominis; and in the lumbosacral region the rectus femoris, tibialis anterior and medial gastrocnemius. Further information about the probe placement in each muscle group can be found as supplementary information. Trapezius was also examined but it was not ascribed to any region since it receives both bulbar and cervical innervation (Pu et al. 2008). The exploration was performed bilaterally in all muscles except the tongue; therefore, altogether, 19 muscles were studied in each patient. Each muscle was observed for 30 seconds and fasciculations, defined as elsewhere (Misawa et al. 2011), were categorized as focal or multifocal. Moreover, the number of fasciculations in each muscle group was counted and the total number of fasciculations was calculated in each individual. A maximum value of 30 fasciculations was assigned to those muscles showing uncountable continuous multifocal fasciculations.

Images of all the 527 limbs muscles were acquired and their muscle thickness was measured with electronic callipers. The thickness of the biceps brachialis, rectus femoris, and tibialis anterior was measured as previously described (Martínez-Payá et al. 2017a). The thickness of the triceps brachialis was measured between the uppermost part of the bone echo of the humerus and the superficial fascia of the triceps. Finally, for the APB and medial gastrocnemius muscles, the thickness was measured including both the superficial and deep epimysiums..

2.4 Electromyography

In 36 patients an EMG study performed with a Dantec Keypoint equipment was accessible in a period not exceeding 30 days (median time 0.16 months [0, 1]). The presence of fasciculations, as well as acute and chronic denervation in each studied muscle were recorded, but this study was performed for diagnostic purposes following consensus criteria for ALS diagnosis (de Carvalho et al. 2008). Briefly, the presence of fasciculations and acute (fibrillations or positive sharp waves, fib-sw) and chronic denervation was assessed by inserting the EMG needle in at least 5 different points and for at least 90 seconds in each muscle group. Acute denervation was considered to be present in a muscle group when fib-sw, persisting at least 2 seconds after the needle insertion, were registered in at least two insertion points. Chronic denervation was considered to be present in a muscle group when increases in the duration, amplitude and phases in at least 3 motor unit potentials together with a decreased interference pattern were found. Most muscles were studied unilaterally and not all muscles were studied in all patients. A total of 263 muscles were studied in the whole cohort (supplementary table 1).

2.5 Statistical analysis

Data were summarized by mean, standard deviation, median, and first and third quartiles for the continuous variables, and by relative and absolute frequencies for the categorical variables. A heat map was used to describe the effect of the symptoms' onset site on the fasciculations pattern. An exploratory analysis was performed by Spearman's correlations to evaluate potential associations of the demographical, clinical and analytical variables with the total number of fasciculations.

Since, both BMI (r=-0.441, p=0.003) and BMI loss (r=0.424, p=0.004), but not the premorbid BMI (r=-0.208, p=0.17), correlated with the number of fasciculations, two multivariable mixed negative binomial regression model were carried out to assess their respective association with the fasciculations' number. In the first model, age, sex, BMI loss, region of onset, disability (ALSFRS-R score) and the degree of UMN (UMN score) and LMN impairment (MRC and muscle thickness) were analysed. We hypothesized that the

association of the muscle strength with the fasciculations' number would be non-linear, since previous EMG studies have shown that the fasciculations' rate and frequency increase with the initial LMN impairment (Mills 2010; Krarup 2011), but they decrease in severely denervated and weak muscle (Krarup 2011; de Carvalho and Swash 2016). Consequently, a quadratic term was adjusted for MRC. Finally, the effect of muscle thickness on fasciculations was thought to depend on the muscle strength so an interaction between both variables was considered in the model. The estimated effects resulting from this model equation were used to predict the estimated number of fasciculations as well as their 95% credibility intervals using the package brms (version 2.2.0).

In the second model, BMI and creatinine (an indirect measure of muscle mass(van Eijk et al. 2018)) were introduced as covariables and BMI loss, MRC and muscle thickness were excluded to avoid collinearity issues between BMI and BMI loss and creatinine with MRC and muscle thickness. The following are additional specifications that were introduced in both models to better fit our assumptions:

1. Given that a maximum value of 30 fasciculations was assigned to those muscles showing uncountable continuous multifocal fasciculations, we included an upper bound set at 30 for censored data, in the model.
2. Since we did not anticipate there was any consistent right-left side difference between individuals, these models were extended with the variable "Side" nested to "Patient" as a random effect with random intercept in order to take dependency among observations into account. Likewise, because the observations from the same muscle are more likely to have a similar number of fasciculations than those from other muscles, and these were considered a sample of the human body muscles, another random effect for "Muscle" was introduced as a random intercept. In the first model, the variance in fasciculations number between the selected muscles was 0.87 CI95% [0.44, 1.77] and the variance between patients was 0.74 CI95% [0.55, 0.94]
3. Weak informative priors for the coefficients of the fixed effects were set in both models: N(0,10), as well as a Cauchy (0,2) was set for the standard deviation of

random effects. A sensitivity analysis using less informative priors (N(0,20) and Cauchy (0,5)) was performed to assess the influence of the chosen priors in the final estimates of the models.

4. Given the degree of complexity required to carry out the models, a more flexible framework was needed and, therefore, a bayesian approach was used.

95% Confidence Intervals (95% CI) are provided for all estimates and a marginal effect plot was performed to ease the interpretation of the interaction between muscle thickness and strength.

The number of muscles showing fasciculations as per US and EMG was compared with a X^2 test. For this comparison, the same muscle groups on the same side were selected in both EMG and US. The differences in the fasciculations' number in each muscle depending on the presence or absence of acute or chronic denervation was assessed with a mixed negative binomial regression model, accounting for the aleatory effects of muscle and individual.

All statistical analyses were performed by an experienced biostatistician (VFF) using R software (version 3.4.3) and clickR (version 0.3.35) packages.

2.6 Ethical approval

The study was approved by the Ethics Committee for Biomedical Research of the La Fe Hospital (Valencia). All the participants gave written informed consent.

3. RESULTS

3.1 Study population

The study included 44 patients and their demographic and clinical characteristics are summarized in Table 1. Eleven patients were classified as LMN-ALS and three as UMN-ALS.

3.2 Fasciculations' characteristics

US was performed in 835 muscles, instead of the expected 836, because one patient had his left hand amputated previously and this APB could not be studied.

Fasciculations were found in at least one region in all patients (although other LMN signs were lacking in 3 patients), and in at least 3 regions in 73% of patients (Table 1). The mean number of fasciculations was similar in cALS patients (125 [84]), LMN-ALS patients (121 [91]) and UMN-ALS patients (115 [122]).

Table 2 summarizes the frequency and number of fasciculations per muscle and region. Fasciculations were detected most frequently in muscles of the limbs, especially in the cervical region, and biceps brachialis was, by far, the muscle with the greatest number and frequency of fasciculations. Fasciculations were not detected in the bulbar region in 16 patients (2 of bulbar onset), but were visible in trapezius in 10 of them (one of bulbar onset). Conversely, fasciculations were present in the tongue in 4 out of the 10 patients not showing fasciculations in trapezius.

Table 3 and Figure 1 represent the frequency and number of fasciculations in each muscle according to the region of onset and show that fasciculations are more frequent in cervical muscles independently of the region of onset. Moreover, fasciculations were overall more frequent and widespread in upper limb (UL) onset patients (Figure 1 and 2). Conversely, fasciculations were equally frequent in the ipsilateral and contralateral side of onset (OR 1.08 [0.87, 1.35], p=0.46).

Of the 263 muscles studied with EMG, acute or chronic denervation was found in 183 (82.9%). Overall, US detected fasciculations more frequently than EMG (79.6% vs 51.9%, p<0.001) (Supplementary Table 2).

3.3 Determinants of fasciculations

In the first model (Table 4), the multivariable analysis confirms that UL onset patients have more fasciculations than LL onset patients. Moreover it shows that cervical and proximal muscles fasciculate more frequently than lumbar and distal ones respectively, independently of other covariables (Figure 3). The model also showed a direct association of fasciculations with the BMI loss, UMN impairment and muscle thickness as well as an inverse association with disability (ALSFRS-R). Finally, a non-linear association with muscle strength and an interaction between muscle strength and thickness was found (Figure 4).

To further analyse the relationship between fasciculations and the LMN impairment, we assessed the differences in the fasciculations' number in denervated vs non-denervated muscles. Fasciculations were more frequent in muscles that showed acute or chronic denervation per EMG than in non-denervated muscles (83.9% vs 55.6%; exp(Estimate)=2.33 [1.43, 3.77], p=0.001).

3.4 Association of fasciculations and BMI

The first model showed that the association of fasciculations and BMI loss was independent of other muscular variables (muscle strength or thickness). Therefore, we hypothesized that this BMI loss would be independent of the muscle mass loss. To test this hypothesis we studied the association between fasciculations and BMI using creatinine, a proxy of the muscle mass (Chiò et al. 2014), as a covariable. The second model confirmed previous associations and showed that BMI was negatively associated with fasciculations (0.86 [0.81, 0.92]) independently of creatinine (Table 5). Moreover, in this model, bulbar onset was also associated with fewer fasciculations than UL onset (0.52 [0.29, 0.87]).

4. DISCUSSION

Fasciculations are a hallmark of ALS and have been linked, in previous electrophysiological studies, to excitability changes in the LMN and UMN (Iwai et al. 2016;

de Carvalho et al. 2017). Our study confirms the US potential to deepen on the clinical and pathophysiological meaning of fasciculations (Noto et al. 2018).

4.1 Where are fasciculations more frequent?

Our study shows that fasciculations are frequent, multifocal and widespread (beyond the region and side of onset) at diagnosis in most ALS patients, as previously shown with the LMN and UMN impairment (Ravits et al. 2007; Krarup 2011; Vázquez-Costa et al. 2018). The distribution and frequency of fasciculations, predominating in the UL muscles and proximal vs distal muscles, confirms previous findings (Krarup 2011; Misawa et al. 2011; Noto et al. 2017, 2018; Tsuji et al. 2017) and has been shown specific for the ALS diagnosis (Tsuji et al. 2017). Here we show that this muscle preference is independent of other factors such as the UMN score and muscle strength or thickness, i.e. of the degree of UMN and LMN impairment. Interestingly, UL onset patients were also those with the greatest number of fasciculations, followed by bulbar and lower limb onset patients. A previous study showed similar, although not statistically significant, results (Tsuji et al. 2017).

Fasciculations were detected in the tongue in 63.6% of patients, including 83.3% of the bulbar onset patients. This confirms the utility of US for fasciculations' detection in the tongue (Misawa et al. 2011). Conversely, fasciculations in cricothyroid were scarce. The detection of fasciculations among cranial nerve innervated muscles has been found to be highly variable (O'gorman et al. 2017). However, the lack of fasciculations in cricothyroid is intriguing because it is clinically impaired in ALS patients. Moreover, the nucleus ambiguous, as happens with the hypoglossal nucleus, shows a severe pathologic involvement in ALS patients (Brettschneider et al. 2013) and receives bilateral direct CM projections from motor cortex (Eisen et al. 2017).

Trapezius has been proposed as an alternative muscle to the tongue for the detection of denervation and fasciculations in the bulbar region (Sonoo et al. 2009). However, in most individuals, trapezius has both bulbar and cervical innervation (Pu et al. 2008). In our study, fasciculations in trapezius were found in 62.5% of patients without fasciculations

in the tongue. Considering the high sensitivity of US (83%) to detect fasciculations in the bulbar region in bulbar onset patients, our study suggests that the presence of fasciculations in trapezius frequently represents cervical rather than bulbar impairment. In fact, in 40% of patients showing fasciculations in tongue, they were lacking in trapezius. Moreover, while fasciculations in bulbar onset patients were more frequent in the tongue than in trapezius, in UL onset patients this predominance pattern was inverted (Figure 1).

4.2 Which demographic factors are associated with fasciculations?

Fasciculations are not associated with demographic factors such as age, sex or premorbid BMI. Conversely, we demonstrate for the first time that fasciculations associate with BMI loss. Two hypotheses, which are not mutually exclusive, could explain this association. First, that fasciculations result in increased energy expenditure. Second, that fasciculations act just as a marker of the extent of the LMN impairment and associate with weight loss due to muscle atrophy. In the latter hypothesis, BMI loss would largely depend on muscle mass loss. However, in our second model, the association of fasciculations with BMI was found to be independent of creatinine, a proxy of the muscle mass (Chiò et al. 2014).

This reinforces the first hypothesis, suggesting that fasciculations, a feature of hyperexcitability, are actually a source and not a bystander of the weight loss. Also in ALS animal models, fasciculations have been proposed to cause an increase of energy expenditure, which could ultimately lead to motor neuron degeneration due to metabolic stress (Dupuis et al. 2011; Vandoorne et al. 2018). This link between hyperexcitability, fasciculations and energy expenditure leading to weight loss and ultimately to motor neuron degeneration could explain that all these factors have been linked to poor prognosis in ALS (Krarup 2011; Marin et al. 2011; Paganoni et al. 2011; Shimizu et al. 2014; Shibuya et al. 2016; Noto et al. 2018; Steyn et al. 2018).

4.3 Which clinical factors are associated with fasciculations?

Two types of fasciculations have been previously described in different disease stages, according to the EMG characteristics (de Carvalho et al. 2017), and both have been linked with hyperexcitability either in the cortex or in the LMN (de Carvalho et al. 2017; Eisen et al. 2017; Noto et al. 2018). Our study confirms that both the UMN and LMN impairment contribute independently to the occurrence of fasciculations. Moreover, it shows limb and muscle-specific variations in the number of fasciculations that are independent of the other clinical or ultrasonographical studied factors and probably reflect divergences in the excitability of the different innervating motor neurons.

4.3.1 UMN impairment

Our finding of a direct and independent contribution of UMN impairment to the rise of fasciculations in ALS is in line with previous studies that have found a supraspinal origin of some fasciculations (de Carvalho et al. 2017), which have been related to central hyperexcitability (Noto et al. 2018). Conversely, our study and others (Higashihara et al. 2012; Tsuji et al. 2017) show that fasciculations are equally frequent despite the lack of overt UMN signs, but are somewhat less frequent in the absence of LMN impairment (e.g. in the primary lateral sclerosis) (de Carvalho and Swash 2016). This suggests that, although the UMN impairment can cause fasciculations, it is not its only source in ALS patients at diagnosis.

4.3.2 LMN impairment

Several consecutive muscle changes are thought to arise throughout the course of ALS. Fasciculations appear early on the disease, before the emergence of LMN loss (Iwai et al. 2016; de Carvalho et al. 2017). Later, the LMN loss begins, but it is initially compensated by collateral sprouting and reinnervation changes (chronic denervation), which are visible electromyographically at that moment, together with fasciculations arising distally in the LMN (de Carvalho et al. 2017). With the disease progression, acute denervation signs may appear due to the inability of collateral sprouting to compensate for the loss of motor neurons (Krarup 2011). When more than a third of LMN have degenerated, mild muscle weakness becomes evident (Wohlfart 1958), which is usually followed by a decrease in

muscle thickness (Arts et al. 2011). Previous EMG studies suggest that, while both the axonal hyperexcitability (Iwai et al. 2016) and the fasciculations' rate and frequency increase with the initial LMN impairment (Mills 2010; Krarup 2011), they decrease in severely denervated and weak muscles (Krarup 2011; de Carvalho and Swash 2016). Our results (Figure 4) fit this pathophysiologic model, showing an initial increase of fasciculations with denervation and mild weakness (attributable to the progressive recruitment of hyperexcitable motor units), which is followed by a late decrease with progressive atrophy and moderate to severe weakness, attributable to a progressive reduction of both motor units and axonal excitability (Krarup 2011; de Carvalho and Swash 2016).

Interestingly, according to our model, the number of fasciculations in thicker muscles peaks later than in thinner ones (Figure 4). Since muscle cross-sectional area has not been found to influence the number of fasciculations (Noto et al. 2017), other factors should explain these differences. The thinnest studied muscles in our study were APB and gastrocnemius, which frequently show acute and chronic denervation in ALS, in comparison to thicker and more proximal muscles such as biceps or quadriceps, which show more frequently fasciculations (Krarup 2011; Higashihara et al. 2012; Babu et al. 2017; Noto et al. 2018). If the cortical input (Eisen et al. 2017) explains this characteristic denervation/fasciculation muscle pattern found in ALS, deserves further investigation. However, what seems clear from our and previous works (Krarup 2011; Higashihara et al. 2012; Babu et al. 2017) is that muscles becoming preferentially denervated suffer an earlier decrease of fasciculations, probably due to an earlier loss of hyperexcitable motor units..

4.3.3 Disability

Finally, the inverse association of fasciculations with disability is compatible with the common observation that fasciculations are an early sign in ALS patients that disappear with disease progression, probably also as the result of the loss of hyperexcitable motor units.

4.4 Strengths and limitations

The main strength of our study is its design with a systematic collection of demographic, clinical and analytical variables in a cohort of patients at an early disease stage and of electrophysiological and ultrasonographical variables in a great pre-specified number of muscles in each patient. The main limitation of the study is that the origin of fasciculations (UMN or LMN) cannot be distinguished by US and consequently, the fasciculations' number is probably the sum of both kinds of fasciculations. However, the statistical model, based on a predefined hypothesis, allows capturing the complexity of the fasciculations' pathophysiology in ALS and the independent contribution of each studied variable. Another limitation is that the premorbid weight was not available in the medical record in every patient. However, only mild discrepancies between the recalled and recorded weight were found in those patients with available records. Consequently, this should not significantly impact our results and conclusions. Furthermore, our study was not specifically designed to compare the sensitivity of US vs. EMG for fasciculations detection, since the study protocol was only applied to the US measurements, whereas EMG was performed as a routine for diagnostic purposes. However, our results are similar to previous studies showing that US is more sensitive than EMG for fasciculations' detection (Walker et al. 1990; Misawa et al. 2011; Higashihara et al. 2012). Finally, the UMN burden was measured only clinically by means of the UMN score, and no biomarker of UMN impairment was used. In some ALS patients with severe LMN impairment, the UMN signs can be masked by the LMN impairment. However, our patients were all at an early stage of the disease and the degree of LMN impairment was overall mild to moderate, making less probable this masking phenomenon. Moreover, the same experienced neurologist performed the examinations, minimizing the variability of the assessment.

4.5 Conclusion

Our study suggests a link between fasciculations and BMI loss, which could be mediated by hyperexcitability. Although fasciculations are driven by both UMN and LMN impairment, they decrease with increasing disability and weakness, probably as a

consequence of the loss of hyperexcitable motor units. Nonetheless, there are limb and muscle-specific variations in the frequency of fasciculations that are independent of these factors. We suggest that, differences in fasciculations rates within and between muscles could be explained by the different degrees of LMN impairment. If this is the result of a variable degree of corticomotoneuronal input, will deserve further investigation.

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Table 1. Demographic, clinical and analytical characteristics of ALS patients at recruitment. [a]Clinical and/or neurophysiological LMN impairment as per Awaji criteria. BMI: body mass index; LMN: lower motor neuron; UMN: upper motor neuron; US: ultrasound.

Variable	n (%)	Mean (SD)	Median (1st,3rd Q)
Age	__	65.37 (10.49)	67.99 (57.52, 72.41)
Male sex	24 (54.5%)	__	__
BMI	__	26.10 (3.92)	25.71 (23.57, 28.26)
Premorbid BMI	__	28.19 (4.11)	28.17 (24.95, 30.1)
BMI loss (%)	__	7.13 (7.93)	4.86 (0.57, 11.95)
Time from disease onset (months)		11.65 (7.82)	8.52 (5.77, 14.31)
Time from diagnosis (months)	__	0.63 (0.92)	0.47 (0, 1.32)
ALSFRS-R Score	__	39.84 (4.8)	42 (36, 43)
UMN Score	__	4.48 (5.04)	2 (0, 8.25)
Region of onset			
Bulbar	12 (27.3%)	__	__
Upper limbs	14 (31.8%)	__	__
Lower limbs	18 (40.9%)	__	__
Creatinine (mg/dL)	__	0.73 (0.16)	0.74 (0.61, 0.81)
Regions with LMN impairment[a]			
0	3 (6.8%)		
1	3 (6.8%)		
2	13 (29.5%)		
3	18 (40.9%)		
4	7 (15.9%)		
Regions with fasciculations (as per US)			
1	1 (2.3%)	__	__
2	10 (22.7%)	__	__
3	15(34.1%)	__	__
4	18 (40.9%)	__	__

Table 2. Muscle characteristics as per ultrasound. ABP: abductor pollicis brevis. [a]The US was performed bilaterally in all muscles except the tongue.

Region and muscle	MRC	Muscle Thickness	Muscles with fascicultations[a]	Type of fasciculations		Number of Fasciculations
	Mean (SD)	Mean (SD)	n (%)	Focal (%)	Multifocal (%)	Mean (SD)
Bulbar	--	--	**35 (26%)**	**28%**	**72%**	--
Tongue	--	--	28 (64%)	21%	79%	18.3 (12.36)
Cricothyroid	--	--	7 (8%)	86%	14%	1.71 (0.76)
Trapezius	--	--	**58 (66%)**	**45%**	**55%**	**11.4 (12.30)**
Cervical	--	--	**220 (83%)**	**21%**	**79%**	--
Biceps brachialis	4.8 (0.62)	23.2 (6.19)	81 (92%)	12%	88%	17.2 (11.43)
Triceps brachialis	4.8 (0.39)	28.6 (6.01)	71 (81%)	23%	77%	12.6 (10.62)
APB	4.2(0.89)	7.2 (2.53)	69 (79%)	31%	69%	9 (8.84)
Thoracic (rectus abdominis)	--	--	**49 (56%)**	**37%**	**63%**	**10.8 (12.69)**
Lumbosacral	--	--	**157 (59%)**	**37%**	**63%**	--
Rectus femoris	4.8 (0.46)	20.1 (5.23)	69 (78.41%)	36%	64%	10.2 (10.18)
Tibialis anterior	4.3 (1.41)	22.4 (3.85)	70 (79.55%)	40%	60%	6.7 (7.76)
Gastrocnemius	4.8 (0.67)	13.6 (2.53)	57 (65%)	35%	65%	7.1 (7.58)

Table 3. Percentage of regions showing fasciculations according to the region of onset. All patients showed fasciculations on the cervical region irrespective of the site of onset. Conversely, two bulbar onset patients did not show fasciculations in the bulbar region. Of those, one patient, who had been classified as pseudobulbar palsy, showed fasciculations in US (without acute or chronic denervation in EMG) in the cervical and lumbar region but not in trapezius. The other patient showed widespread acute and chronic denervation as well as fasciculations in both US and EMG, including in trapezius. Finally, only one lower limb onset patient did not show fasciculations in the lumbar region, but did it in the bulbar and cervical regions. LL: lower limbs; UL: upper limbs; EMG: electromyography; US: ultrasound.

	Region of onset		
Anatomical region	Bulbar (n=12)	UL (n=14)	LL (n=18)
Bulbar, n (%)	10 (83.3%)	10 (71.4%)	8 (44.4%)
Cervical, n (%)	12 (100%)	14 (100%)	18 (100%)
Lumbar, n (%)	12 (100%)	13 (92.9%)	17 (94.4%)

Table 4. Model analysing the association between fasciculations and demographic and clinical variables.
BMI loss, ALSFRS-R and UMN score associate directly with fasciculations, whereas LL onset associates with less fasciculations. Moreover, the model confirms the existence of a non-lineal association between fasciculations and muscle strength and of an interaction between muscle strength and thickness (see also Figure 4). In bold are highlighted those variables showing an independent effect on the fasciculations' number. BMI: body mass index; LL: lower limbs; MRC: muscle strength based on the medical research council scale; MRC^2: quadratic adjustment of MRC; MTh: muscle thickness; UMN: upper motor neuron.

	exp(Estimate)	Credibility Interval 95%	
Age	0.982	0.958	1.006
Male gender	0.748	0.464	1.202
BMI loss	1.074	1.043	1.106
ALSFRS-R	1.074	0.995	1.158
UMN score	1.095	1.036	1.160
Bulbar onset	0.650	0.38	1.104
LL onset	0.420	0.254	0.699
MRC	8.584	2.995	24.882
MRC^2	0.805	0.712	0.908
MTh	1.203	1.077	1.352
MRC:MTh	0.960	0.937	0.982

Table 5. Model analysing the association between fasciculations and demographic and clinical variables. In this model, ALSFRS-R and UMN score associate directly with fasciculations, whereas BMI, and bulbar and LL onset associate with less fasciculations. Those variables highlighted in bold show an independent effect on the fasciculations' number. BMI: body mass index; LL: lower limbs; MRC: muscle strength based on the medical research council scale; MTh: muscle thickness; UMN: upper motor neuron;

	exp(Estimate)	Credibility Interval 95%	
Age	1.015	0.985	1.047
Male gender	0.777	0.475	1.276
BMI	0.860	0.809	0.919
Creatinine	0.323	0.099	1.071
ALSFRS-R	1.076	0.993	1.166
UMN score	1.067	1.013	1.127
Bulbar onset	0.516	0.296	0.871
LL onset	0.335	0.210	0.543

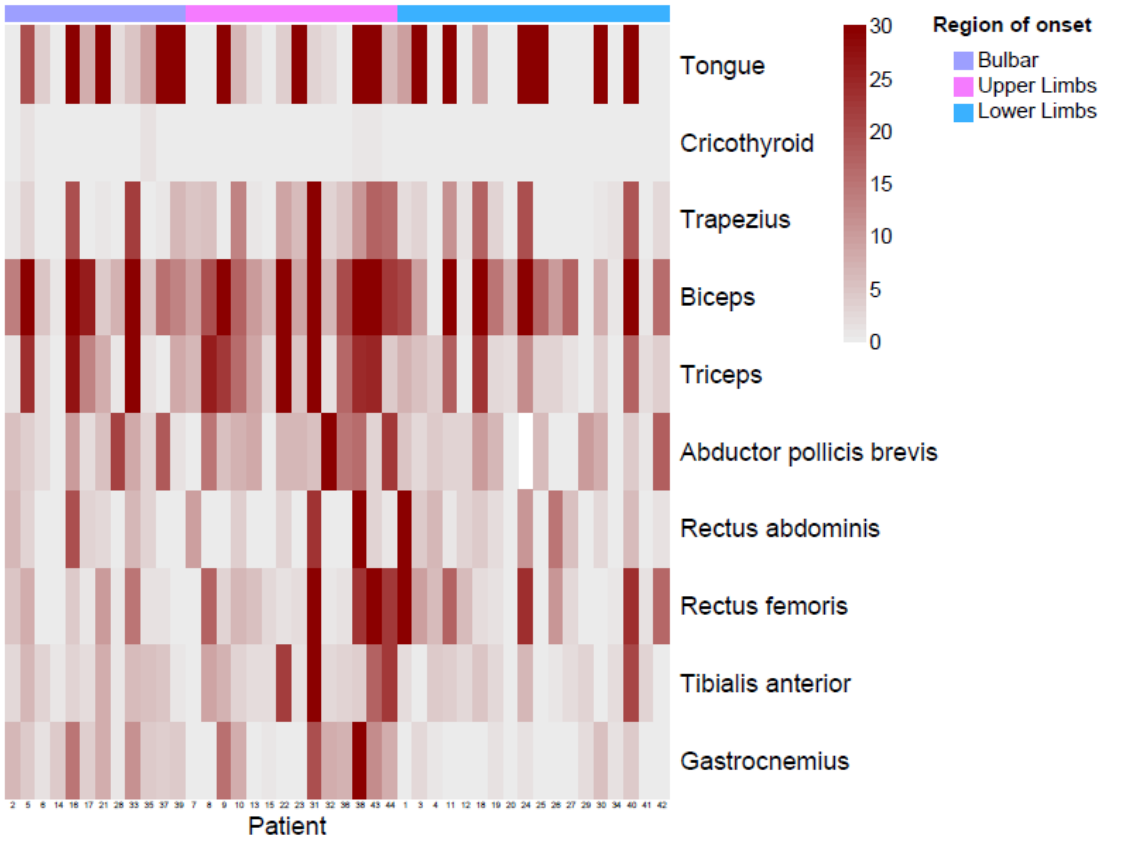

Figure 1. Heat map representing the number of fasciculations in each muscle according to the region of onset.

In bulbar onset patients, fasciculations are less frequent in the thoracic and lumbar region. Patients with onset in the upper limbs show widespread fasciculations, although predominating in the cervical region. Intriguingly, in lower limbs onset patients, fasciculations are less frequent in the distal muscles of the lumbar region. Fasciculations in the tongue seem to be more frequent in bulbar onset patients. Conversely, in trapezius, fasciculations are more frequent in UL onset patients. Fasciculations in the cervical and thoracic regions show little variation regardless of the disease onset, whereas fasciculations in tibialis anterior and especially in gastrocnemius appear to be less frequent in lower limb onset patients. LL: lower limbs; UL: upper limbs.

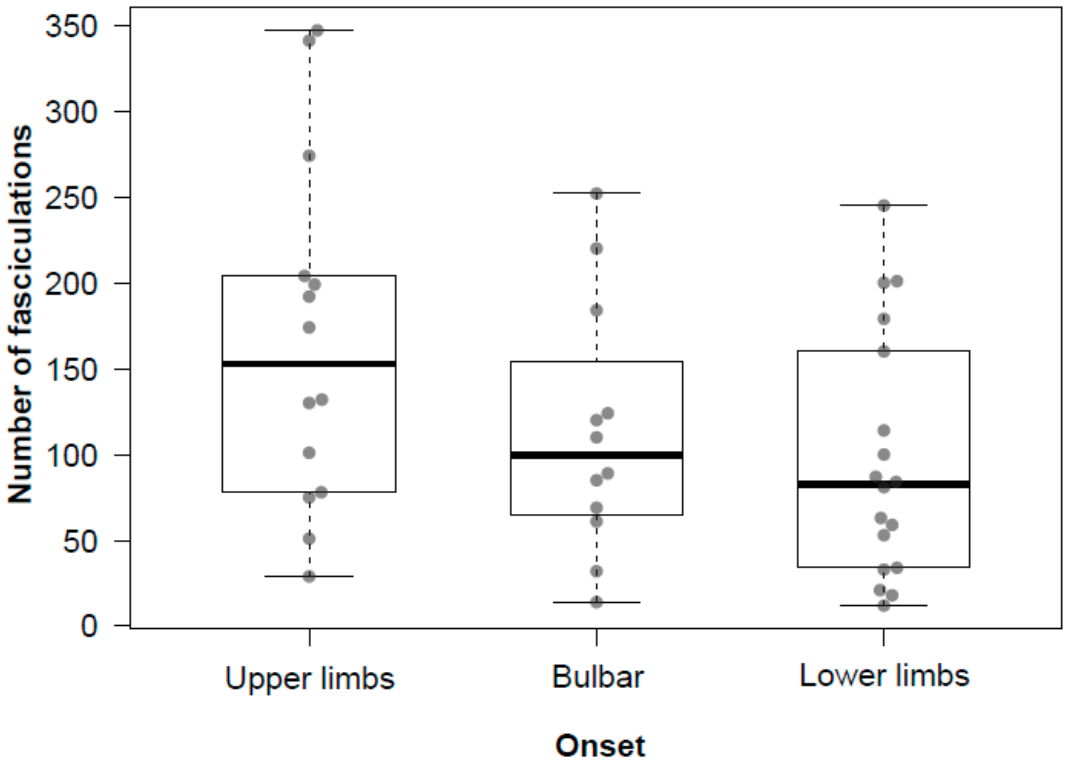

Figure 2. Number of fasciculations per region of onset.

UL onset patients show greater fasciculations than bulbar onset patients and lower limb onset patients.

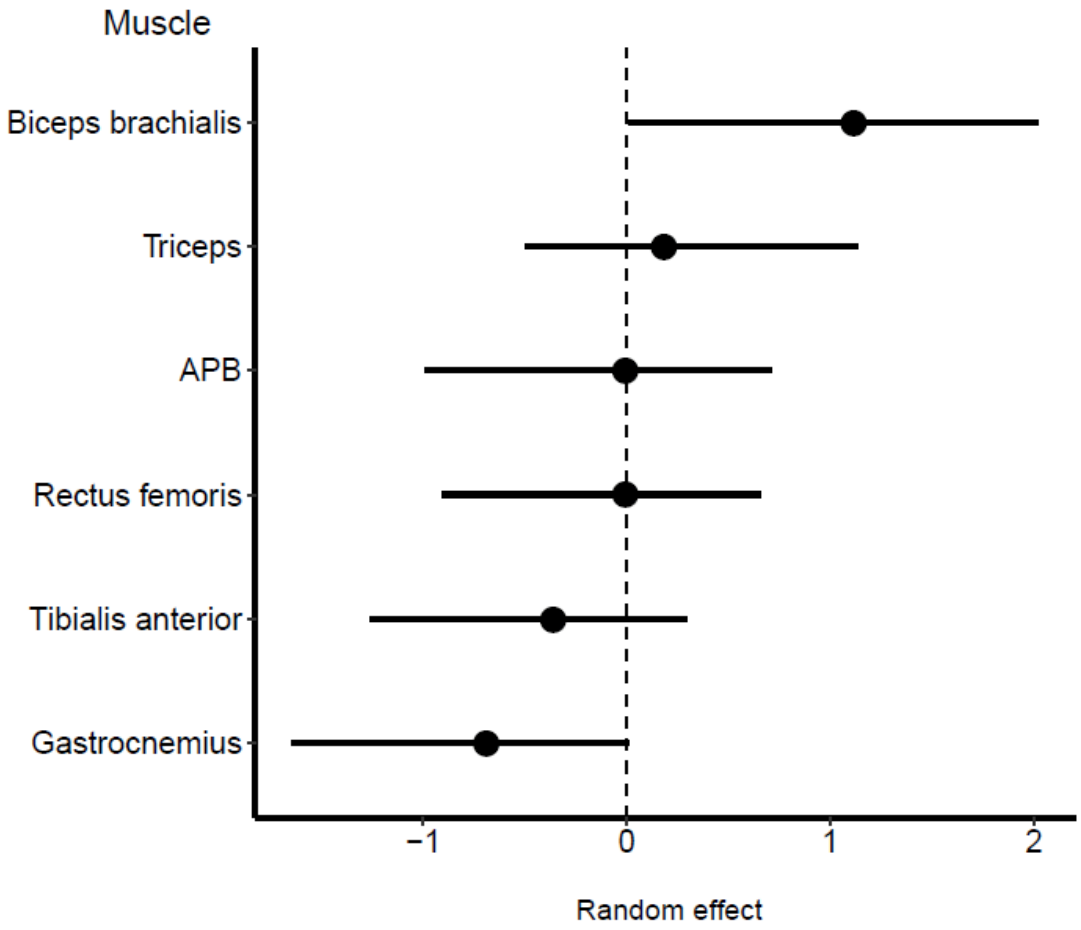

Figure 3. Number of fasciculations in the different muscle groups predicted by the model of Table 3.

Muscles in the graphic are shown in a descendant order based on the number of fasciculations according to the model and after adjusting by other co-variables. In other words, this graphic represents the independent effect of each muscle on the fasciculations number. The graphic was developed considering the variable "Muscle" as a random effect with random intercept to correct for the non-independence of the data. The variance of the fasciculations' number between the selected muscles was 0.867 CI95% [0.44, 1.77]. APB: abductor pollicis brevis.

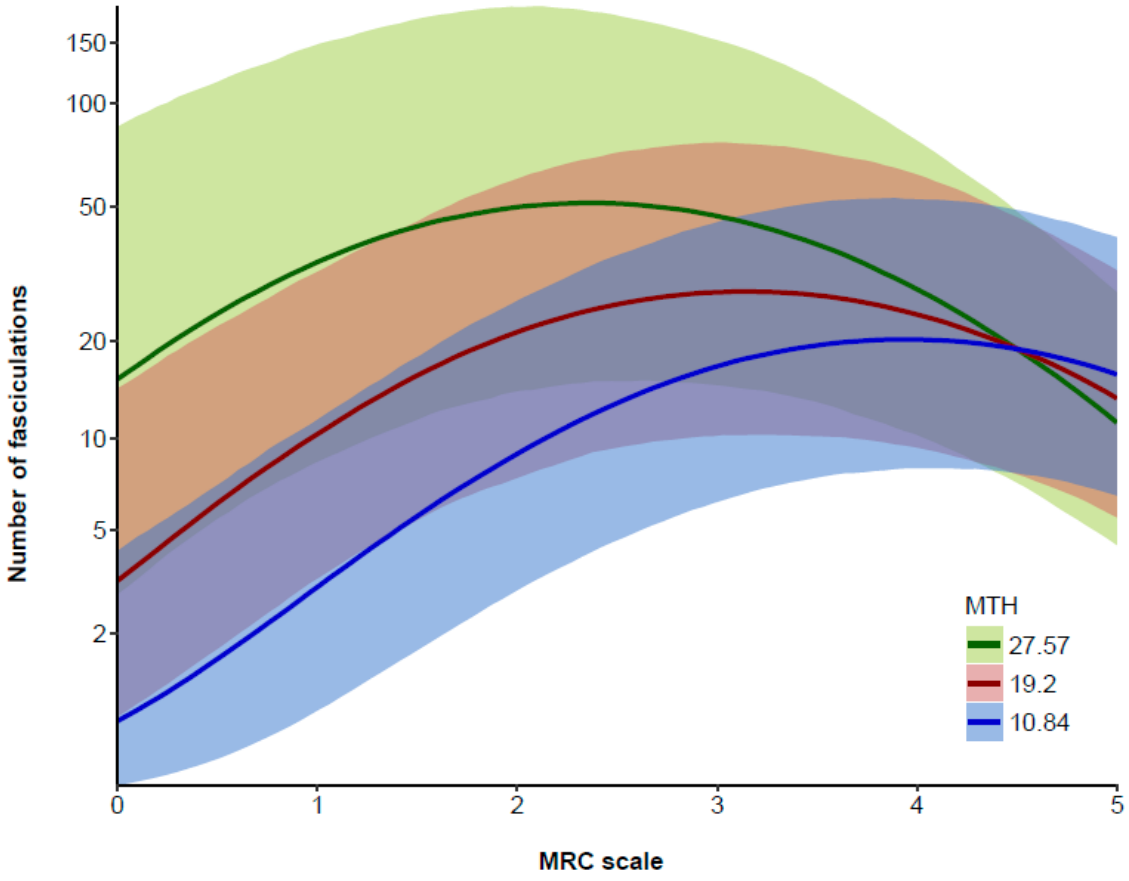

Figure 4. Graphic representation of the association between the fasciculations number and the interaction of muscle strength (MRC) and thickness (MTh) predicted by the model of Table 3.

On the one hand, greater MTh associates with greater fasciculations number (colours). On the other hand, the model shows an inverted U-shape association of the fasciculations' number with muscle strength. Namely, the number of fasciculations initially increases with the loss of muscle strength until a particular point (determined by the MTh), where it progressively decreases. The interaction between muscle strength and thickness means that thicker muscles (such as biceps or triceps brachialis) need to be severely weak to show a decrease in fasciculations, whereas thinner muscles (such as abductor policis brevis) show an early decrease in fasciculations with the onset of weakness.