ANÁLISIS DE BIOMARCADORES DE IMAGEN POR RESONANCIA MAGNÉTICA EN EL INFARTO CEREBRAL

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Grado de Doctor

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Dedicatoria.

A mi mujer Virginia

A mis hijos José e Inés

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Abreviaturas

ACM: Arteria cerebral media

AF o FA: Anisotropía Fraccional.

rAF o rFA: ratio de Anisotropía Fraccional

ARM: Angiografía por Resonancia Magnética.

BPCI: Brazo posterior de cápsula interna.

BRIC: Biomarcadores radiológicos en el infarto cerebral.

BRIC-RM: BRIC por RM

CR: Corona radiata.

DE: Desviación estándar

DW: Degeneración Walleriana.

FLAIR: Atenuación de líquido con inversión recuperación.

GE: Eco de Gradiente.

ITD: Imagen del tensor de Difusión.

MTT: Tiempo de tránsito medio.

NIHSS: Escala de ictus del Instituto Nacional de Salud.

m-NIHSS: Ítems motores de la Escala NIHSS.

RM: Resonancia Magnética.

ROI: Región de interés.

rTPA: Activador del Plasminógeno Tisular recombinado.

SC: Área subcortical.

TC: Tomografía computarizada.

TCE: Tracto córticoespinal.

TOF: Tiempo de vuelo.

TTD: Tractografía por tensor de difusión.

VPN: Valor Predictivo Negativo.

VPP: Valor Predictivo Positivo

1. Introducción

El infarto cerebral es una enfermedad frecuente que provoca una gran morbilidad y mortalidad en nuestra sociedad. El único tratamiento aprobado hasta ahora es la terapia con rTPA (1) pero este tratamiento tiene varias limitaciones como una ventana terapéutica corta, el pequeño porcentaje de pacientes a los que se les puede aplicar y el riesgo de transformación hemorrágica (2). Por todo ello es importante aprobar tratamientos mejores que rTPA aunque el proceso de aprobación actual de nuevos fármacos es extremadamente largo (cerca de 12 años) y caro (3).

Para solucionar esta situación se han introducido nuevos biomarcadores que deben sustituir a los marcadores clínicos clásicos basados en la morbilidad y mortalidad del paciente (4). En teoría estos nuevos biomarcadores podrán demostrar el efecto terapéutico más claramente, por lo que serán precisos menos pacientes para poder conseguir demostrar un resultado positivo que permita aprobar estos nuevos tratamientos. Por todo ello estos nuevos biomarcadores podrían ayudar a aprobar nuevos fármacos de una manera más rápida y económica (4).

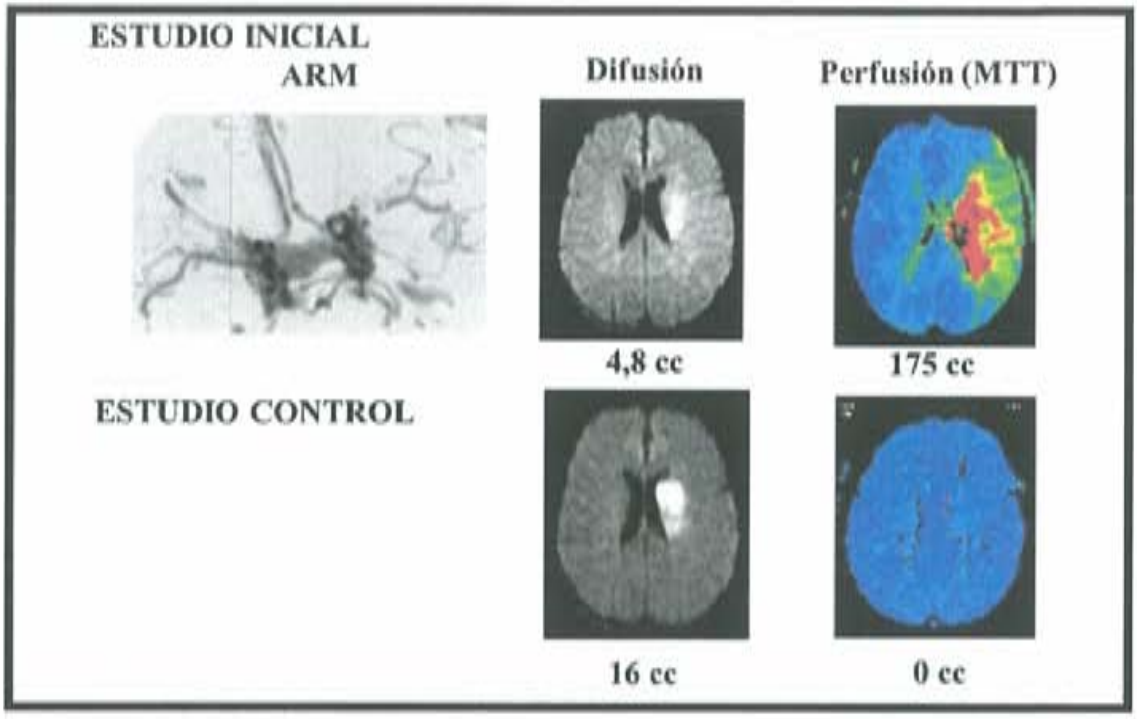
Hasta ahora se han propuesto múltiples marcadores genéticos y moleculares (2, 5, 6,7) y algunos biomarcadores radiológicos (5, 8, 9).

El infarto cerebral se puede estudiar por diferentes técnicas radiológicas como ecografía, tomografía computarizada (TC), angiografía y Resonancia Magnética (RM). La RM es una técnica muy sensible para el diagnóstico precoz del infarto cerebral y

para hacer el seguimiento en fase subaguda-crónica. La RM aporta múltiples variables radiológicas cuantificables pero no está clara cuales serían los biomarcadores válidos.

En la Figura 1, se muestra un ejemplo de cuantificación de biomarcadores radiológicos en un paciente con infarto cerebral. El paciente presentó un ictus con un score de la escala de NIHSS (escala de ictus del instituto Nacional de Salud) de 20. En el estudio inicial la secuencia vascular contrastada mostraba una estenosis severa proximal de Arteria cerebral media (ACM) izquierda con flujo distal. La secuencia de Difusión mostraba un pequeño infarto subcortical de ACM izquierda cuyo volumen era de 4,8 cc. El estudio de perfusión mostraba un trastorno de la perfusión en el mapa de Tiempo de tránsito medio (MTT) a nivel del territorio superficial y profundo de ACM. Su volumen era mucho mayor (175 cc) que el del infarto apreciado en la secuencia de Difusión lo cual indica la existencia de un área de penumbra o de tejido en riesgo de 170,2 cc (diferencia entre difusión y perfusión). El paciente recibió tratamiento trombolítico y se consiguió una recanalización precoz de la oclusión de ACM (no mostrada) sin transformación hemorrágica en la secuencia eco de gradiente (no mostrado). Se apreciaba una normalización completa de la alteración de perfusión (volumen 0) con leve aumento de la lesión en Difusión (volumen de 16 cc). Todo ello asociado a una mejora clínica con reducción del NIHSS a 3

FIGURA 1. Ejemplo de cuantificación de biomarcadores radiológicos en el infarto cerebral por Resonancia Magnética



ARM: Angiografía por Resonancia magnética. MTT: Tiempo de tránsito medio.

Existen amplios estudios sobre los criterios para validar a los biomarcadores moleculares pero hay una necesidad de revisiones similares sobre biomarcadores radiológicos en el infarto cerebral (BRIC). La primera línea de investigación de esta tesis se centra en la validación de estos biomarcadores.

El volumen del infarto cerebral es uno de los biomarcadores radiológicos más usados en los ensayos clínicos (10, 11, 12, 13, 14, 15, 16,17). Se considera que el método planimétrico es el patrón de referencia para cuantificar el volumen del infarto (10, 11,

12) pero tiene el inconveniente de que precisa mucho tiempo para su realización y no se puede realizar en fase aguda. El método ABC/2 por el contrario es muy rápido y se puede realizar en fase aguda aunque tiene el inconveniente de que no estaba determinada su fiabilidad (18, 19). La segunda línea de investigación de esta tesis analiza la fiabilidad de este método ABC/2 para cuantificar el volumen del infarto en la RM en fase aguda.

La pérdida de la función motora es una de las secuelas más comunes de los pacientes con infarto y se correlaciona con invalidez y reducción de la calidad de vida (20, 21). Además un 65% de pacientes pueden mostrar un cierto grado de recuperación de la función motora (22). Todo ello hace necesario la validación de biomarcadores radiológicos predictivos del déficit motor.

Diferentes estudios han demostrado que la recuperación motora depende de la integridad de las fibras motoras siendo el tracto córticoespinal (TCE) el principal tracto responsable de los movimientos motores.

La secuencia de Imagen del tensor de Difusión (ITD) permite visualizar y cuantificar el daño estructural en los tractos de sustancia blanca (23). En primer lugar podemos realizar una tractografía por tensor de Difusión (TTD) con reconstrucción de los diferentes tractos de sustancia blanca (24). En segundo lugar podemos cuantificar directamente parámetros como la anisotropía fraccional (AF) para demostrar la integridad de los tractos de sustancia blanca.

Se ha propuesto la asociación del déficit motor con la existencia de degeneración walleriana del TCE y con una reducción de los valores de AF

(25, 26, 27, 28). Sin embargo existen pocos estudios prospectivos que hayan validado el valor del AF como biomarcador pronóstico.

Es necesario validar biomarcadores radiológicos basados en TTD que puedan predecir cómo será la evolución de los pacientes con infarto. En la tercera investigación de esta tesis se analiza el valor pronóstico que puede tener el nivel de afectación del tractograma del TCE. En la cuarta investigación se valora directamente el valor de la AF para predecir el déficit motor del paciente.

2. Objetivos.

Los objetivos principales de esta tesis son:

1. Establecer el conjunto de posibles biomarcadores radiológicos del infarto cerebral en el estudio por RM determinando los criterios de validación.

2. Analizar la validez de los métodos de cuantificación del volumen del infarto cerebral por RM.

3. Evaluar la validez de la topografía de afectación de tracto córticoespinal en fase aguda del infarto como biomarcador pronóstico de la función motora final del paciente con infarto.

4. Determinar la validez de la AF como biomarcador pronóstico en fase aguda y subaguda de la función motora final del paciente con infarto

Los objetivos específicos de cada investigación de la tesis son:

Investigación de base sobre biomarcadores de imagen

1. Establecer los criterios para poder validar una variable radiológica como biomarcador en el infarto cerebral.

2 Evaluar la validez como biomarcador radiológico de las diferentes variables de un estudio por RM en el infarto cerebral.

Investigación sobre el biomarcador volumen del infarto cerebral

1. Determinar la fiabilidad del método ABC/2 de cálculo del volumen del infarto cerebral agudo en la secuencia de Difusión por RM.

2. Analizar la reproducibilidad del método ABC/2 para cuantificar el volumen del infarto cerebral en la secuencia de Difusión por RM.

Investigación sobre el biomarcador de tractografía

1. Evaluar si el compromiso del TCE en la TTD en una localización especifica permite predecir el daño axonal de la vía motora así como la función motora final.

2. Determinar si un modelo predictivo formado por la información sobre el nivel de afectación del TCE en la TTD junto con la escala motora tiene mayor capacidad predictiva que las escalas clínicas aisladas.

Investigación sobre el biomarcador anisotropía fraccional

1. Evaluar de la correlación de la AF con el grado de déficit motor en pacientes con infarto cerebral en comparación con las escalas clínicas validadas en fase aguda y subaguda.

3. Métodos.

3.1. Población del estudio. Selección de pacientes.

La base de datos de estudios por RM en pacientes con infarto cerebral tiene unos criterios de inclusión y exclusión comunes que se han aplicado en todas las investigaciones de esta tesis:

- Los criterios de inclusión comprendían: Ictus de menos de 12 horas de evolución, edad mayor de 18 años, valoración de Escala de Rankin menor de 2 e infarto de arteria cerebral media.

- Los criterios de exclusión comprendían: Pacientes en coma, síndromes lacunares, accidente isquémico transitorio, infarto cerebral previo que artefacto la valoración clínica y neurológica, enfermedad crónica severa y enfermedad inflamatoria.

Todos los pacientes fueron tratados siguiendo las guías de práctica clínicas publicadas en la literatura. Cada uno de los trabajos fue aprobado por el comité ético del Hospital Dr Josep Trueta de Girona y el paciente o un familiar firmó el consentimiento informado del estudio.

Dado que la tesis tiene por objeto la validación de diferentes biomarcadores, el trabajo se ha desarrollado con diferentes grupos de pacientes de esta base de datos en función del objetivo específico de investigación

Investigación de base sobre biomarcadores de imagen

Dado que el objetivo era definir criterios de validez de biomarcadores y un grupo de biomarcadores en las publicaciones de la literatura no se ha analizado pacientes directamente.

Investigación sobre el biomarcador volumen del infarto cerebral

Se han estudiado 86 pacientes con infarto cerebral.

Investigación sobre el biomarcador tractografía

Se ha estudiado un grupo de 60 pacientes

Investigación sobre el biomarcador anisotropía fraccional

Se ha estudiado un grupo de 60 pacientes

3.2. Variables clínicas.

La base de datos de estudios por RM en pacientes con infarto cerebral recoge una batería de variables clínicas que se han aplicado en las investigaciones de esta tesis:

Un neurólogo senior certificado determinó el valor de NIHSS en tres momentos de la evolución del paciente (día 3, día 30 y día 90).

El subíndice motor m-NIHSS categoriza el déficit motor en tres grados de peor a mejor función motora: Grado I (total m-NIHSS score de 0), Grado II (m-NIHSS, 1-4) y Grado III (m-NIHSS, 5-8).

El índice de Barthel y la escala modificada de Rankin se usaron para medir la incapacidad y dependencia para las actividades de la vida diaria en el día 90 de evolución.

Todos los datos clínicos se determinador sin conocimiento de la cuantificación de los biomarcadores radiológicos.

Dado que la tesis tiene por objeto la validación de diferentes biomarcadores, en algunas líneas de investigación se han analizado variables específicas.

Investigación de base sobre biomarcadores de imagen

Dado que el objetivo era definir criterios de validez de biomarcadores y un grupo de biomarcadores en las publicaciones de la literatura no se han obtenido variables clínicas directamente en un grupo de pacientes.

Investigación sobre el biomarcador volumen del infarto cerebral

En esta investigación de correlación de datos radiológicos no se analizaron variables clínicas concretas.

Investigación sobre el biomarcador tractografía

En esta investigación se determinaron las escalas clínicas generales comentadas.

Investigación sobre el biomarcador anisotropía fraccional

En esta investigación se determinaron las escalas clínicas generales comentadas.

3.3. Protocolo radiológico.

La base de datos de estudios por RM en pacientes con infarto cerebral tiene un protocolo radiológico común que se han aplicado en las investigaciones de esta tesis.

Las características del protocolo radiológico son:

- Los estudios de RM se realizan en un equipo de 1.5 Teslas.

- El protocolo radiológico incluía las siguientes secuencias: Difusión, FLAIR (Atenuación de líquido con inversión recuperación), T2-GE (Eco de Gradiente), Perfusión, Angiografía por RM (ARM) con técnica TOF (Tiempo de vuelo) y secuencia ITD (Imagen de Tensor de Difusión). Los parámetros técnicos se detallan en los artículos.

- El protocolo radiológico se repetía en tres fases de la evolución del paciente: el estudio del primer día, el control del tercer día y el control del día 30 de evolución

Investigación de base sobre biomarcadores de imagen

Dado que el objetivo era definir criterios de validez de biomarcadores y un grupo de biomarcadores en las publicaciones de la literatura no se ha obtenido estudios radiológicos en un grupo de pacientes.

Investigación sobre el biomarcador volumen del infarto cerebral

En esta investigación se realizó el protocolo radiológico común.

Investigación sobre el biomarcador tractografía

En esta investigación se realizó el protocolo radiológico común.

Investigación sobre el biomarcador anisotropía fraccional

En esta investigación se realizó el protocolo radiológico común.

3.4. Postproceso radiológico.

Dado que la tesis tiene por objeto la validación de diferentes biomarcadores, el trabajo se ha desarrollado con diferentes técnicas de postproceso en función del objetivo específico de cada una de las investigaciones de la tesis.

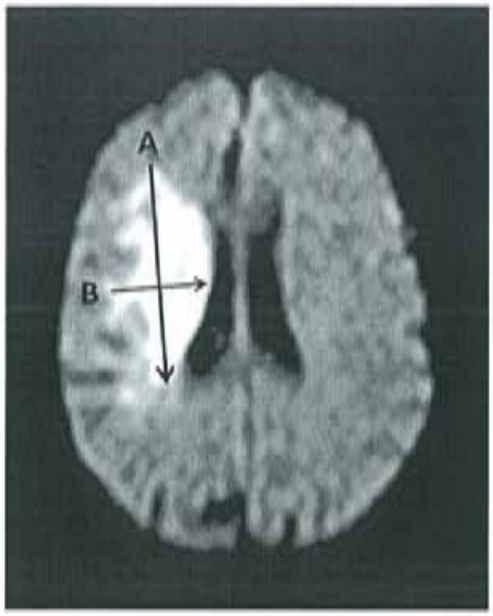
Investigación de base sobre biomarcadores de imagen

Dado que el objetivo era definir criterios de validez de biomarcadores y un grupo de biomarcadores en las publicaciones de la literatura no se ha realizado postproceso radiológico directamente.

Investigación sobre el biomarcador volumen del infarto cerebral

Tres observadores de manera ciega determinaron el volumen del infarto cerebral con la técnica ABC/2 y con el método planimétrico.

FIGURA 2. Método ABC/2,



La figura 2 muestra un ejemplo de aplicación del método ABC/2. Primero se escoge el corte en el que el infarto tiene el mayor volumen y se determina el diámetro antero posterior mayor (valor A) y el diámetro perpendicular mayor en el mismo nivel (valor B). Después se determina el diámetro vertical (valor C) mediante la suma del grosor de todos los cortes en que se ve el infarto. El valor del volumen se obtiene entonces con la fórmula A x B x C x 0.5.

Investigación sobre el biomarcador tractografía

Se realizó reconstrucción de la tractografía a partir del estudio de IDT. Las especificaciones técnicas se detallan en el artículo.

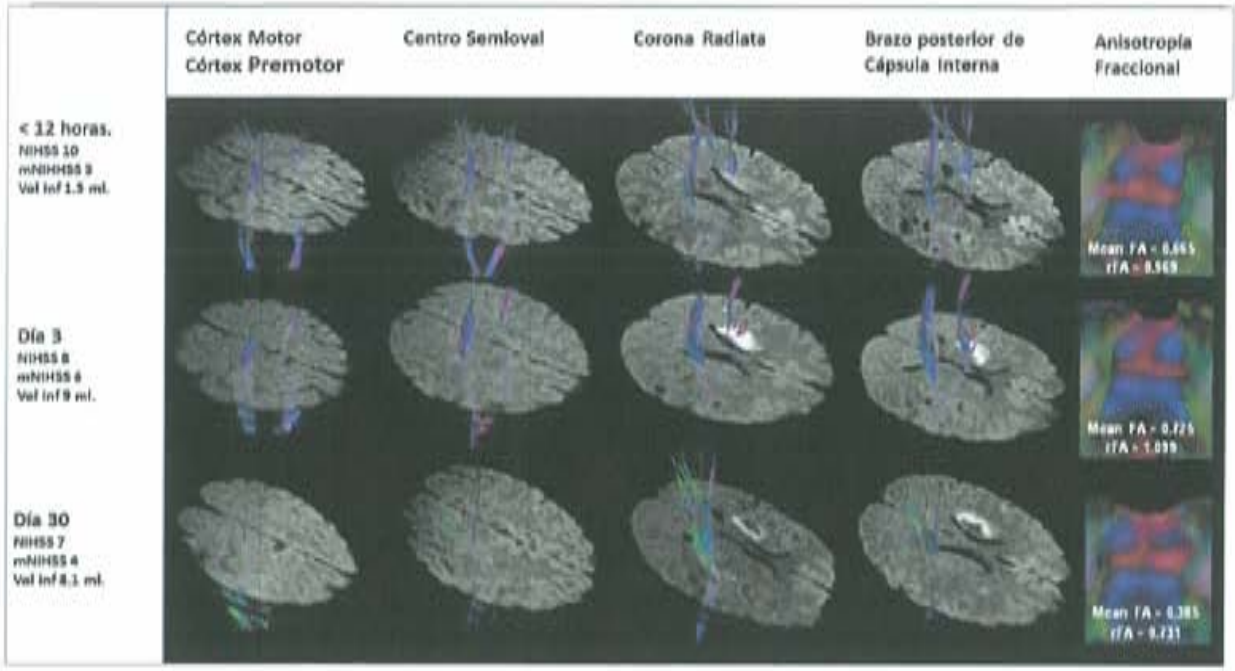
Para determinar que segmento del TCE estaba afectado por el infarto se hizo una superposición de las imágenes de Difusión con el tractograma del TCE. Se analizó la relación con el infarto en 5 niveles: córtex motor y premotor, sustancia blanca subcortical, corona radiata y brazo posterior de cápsula interna (BPCI). Se realizó esta valoración en los estudios del día 1°, 3° y 30.

Se calculó el valor de AF en el bulbo a nivel homolateral o contra lateralmente al lado del infarto. Se determinó también el ratio entre el valor de AF del lado afecto respecto al contralateral.

También se determinó el volumen del infarto mediante el método planimétrico.

La figura 3 muestra un ejemplo de un paciente de 45 años con un infarto pequeño pero con un déficit motor importante dada la afectación del TCE en el BPCI. Se muestra la superposición del TCE en diferentes niveles cerebrales: Córtex motor-premotor, Centro Semioval, Corona radiata y Brazo posterior de Cápsula interna. En el estudio del primer día se aprecia un pequeño infarto de territorio profundo de ACM izquierda. En el estudio de control al día 3 se aprecia el crecimiento del infarto con compromiso del TCE. En el estudio final se ve la ausencia del TCE con una reducción de la anisotropía fraccional a nivel del bulbo indicando la degeneración walleriana de la vía piramidal.

FIGURA 3. Ejemplo de compromiso de vía piramidal en brazo posterior de cápsula interna.



NIHSS: Escala de infarto del Instituto Nacional de Salud, mNIHSS: Ítems motores de la escala, Vol.: Volumen, Inf: inferior, FA: Anisotropía Fraccional, rFA: Anisotropía Fraccional relativa.

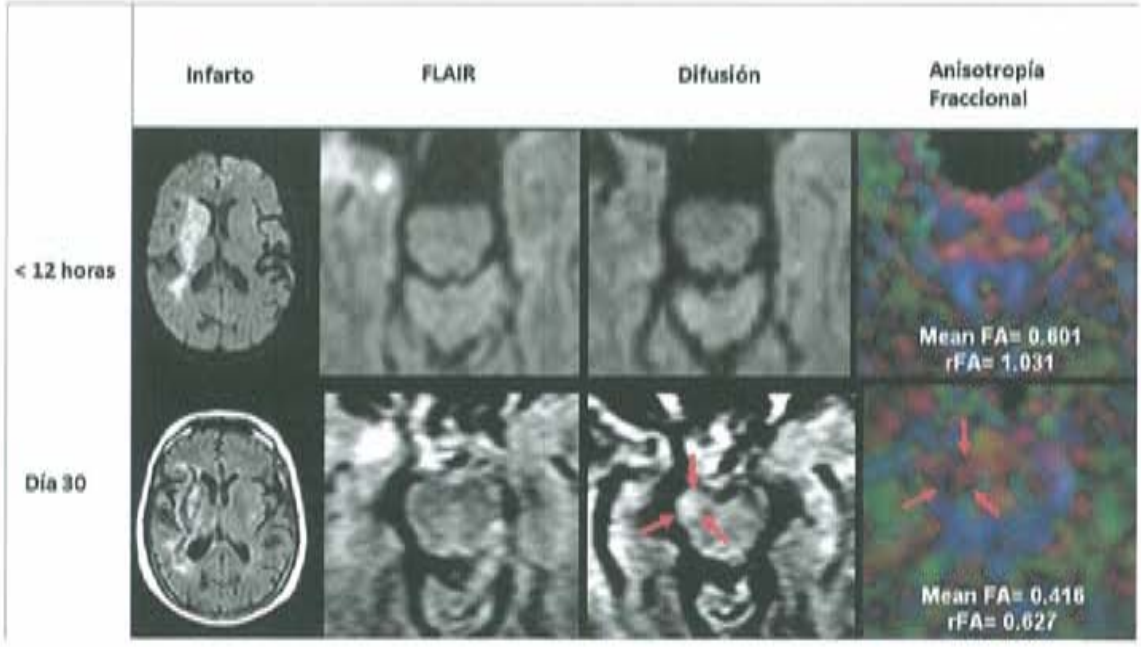
Investigación sobre el biomarcador anisotropía fraccional

Se cuantificó el valor de AF del TCE a nivel del bulbo tanto en el lado homolateral al infarto como en el lado contralateral mediante la colocación de regiones de interés (ROI). Se calculó el ratio rFA como la razón entre el valor de AF del lado afecto con respecto al lado contralateral sano.

La figura 4 muestra un ejemplo de degeneración walleriana en un paciente de 67 años con un infarto de ACM y una hemiparesia severa (mNIHSS de 8). En el estudio inicial

no se aprecia alteración de la señal en el tronco. Sin embargo en el estudio del día 30 se aprecia una hiperintensidad focal en la mitad derecha de la protuberancia (indicado por flechas) y una reducción de la señal en la misma área en el mapa de AF. Todo ello son signos de la Degeneración walleriana del tracto cortico espinal derecho.

FIGURA 4. Ejemplo de cambios por RM en la Degeneración Walleriana.



4. Copia de Publicaciones.

4.1. Artículo 1. *Investigación de base sobre biomarcadores de imagen en RM*

Magnetic resonance imaging biomarkers of ischemic stroke: criteria for the validation of primary imaging biomarkers. Drug News Perspect. 2009 Oct; 22(8):481-6.

FOCUS ON BIOMARKERS

MAGNETIC RESONANCE IMAGING BIOMARKERS OF ISCHEMIC STROKE: CRITERIA FOR THE VALIDATION OF PRIMARY IMAGING BIOMARKERS

Further large, randomized trials will enable us to overcome the limitations of current MRI biomarkers of acute ischemic stroke and validate new biomarkers.

by Salvador Pedraza, Josep Puig, Gerard Blasco, Josep Daunis-i-Estadella, Imma Boada, Anton Bardera, Alberto Prats, Mar Castellanos and Joaquín Serena

SUMMARY

Ischemic stroke is associated with a high rate of disability and death. Establishing valid biomarkers could help accelerate the approval of promising new therapies for stroke. Whereas many serum biomarkers have been evaluated, possible imaging biomarkers of stroke lack validation. Magnetic resonance imaging (MRI) is a very sensitive technique to study acute stroke and MRI parameters have been established to assess the outcome of acute stroke. This review reassesses the criteria for the validation of MRI biomarkers of acute ischemic stroke (MRI-BAS). Seven criteria were used to review the validity of the main MRI-BAS: vascular status, lesion volume, reversibility on diffusion-weighted imaging, perfusion alteration, penumbra studied with diffusion–perfusion mismatch, clinical–diffusion mismatch, diffusion–angiography mismatch and hemorrhagic transformation. We analyzed the definitions of these biomarkers and the extent to which each fulfills the criteria for validation and found that few MRI-BAS have been fully validated. Further studies should help to improve the validation of current MRI-BAS and develop new biomarkers.

INTRODUCTION

Ischemic stroke is common and is associated with a high rate of disability and death. Thrombolytic therapy with recombinant tissue plasminogen activator (rt-PA) remains the only treatment proven to be effective in acute ischemic stroke.[1] However, it has important limitations, such as a narrow therapeutic window, the small percentage of patients currently benefiting from this therapy and the risk for significant hemorrhagic transformation. Therefore, it is essential to find new therapies for stroke.[2] Unfortunately, the approval of new treatments is a long (nearly 12-year) and expensive process.[3] To overcome this difficulty, a new infarct definition, based on the presence of cell death in the brain or retina due to cerebral ischemia, was introduced.[4] In addition, the use of new surrogate endpoints or biomarkers [5] was proposed to replace the clinical endpoints of morbidity and mortality. In theory, these surrogate outcomes can detect signs of therapeutic benefit more clearly.[6] Thus, fewer patients would be required to achieve positive results and effective treatments could be found more quickly and economically.

Many genetic and molecular biomarkers [2,6-8] and several imaging biomarkers [6,9,10] have been proposed. Magnetic resonance imaging (MRI) has an established role in the study of acute stroke patients (Fig. 1).[11] Different MRI techniques are useful in the study of acute stroke. Magnetic resonance angiography (MRA) can evaluate a patient's vascular status. Diffusion-weighted imaging (DWI) and T2*-weighted sequences can differentiate between ischemic and hemorrhagic stroke. Combined DWI and perfusion-weighted imaging (PWI) can distinguish between definitively infarcted and potentially salvageable tissue (penumbra). Finally, MRI is useful in follow-up after treatment, detecting changes in lesion size, evaluating recanalization, and determining the grade of hemorrhagic transformation.[12,13] However, to our knowledge, the validation criteria for MRI biomarkers of stroke have yet to be defined.

In this article, we will define the specific requisites for validating MRI biomarkers of acute ischemic stroke (MRI-BAS) and review the extent to which the main MRI biomarkers currently fulfill these criteria.

VALIDATION CRITERIA

Biomarkers of ischemic stroke should have the following utilities: 1) to diagnose ischemic stroke; 2) to identify the stage of the ischemic lesion (e.g., as markers of definitively infarcted or of potentially salvageable tissue); and 3) to predict the evolution of ischemic stroke, both spontaneously and after therapy (e.g., increase of lesion volume, monitoring the response to therapy and its possible secondary effects). Other features of good biomarkers include specificity and sensitivity for a given disease and

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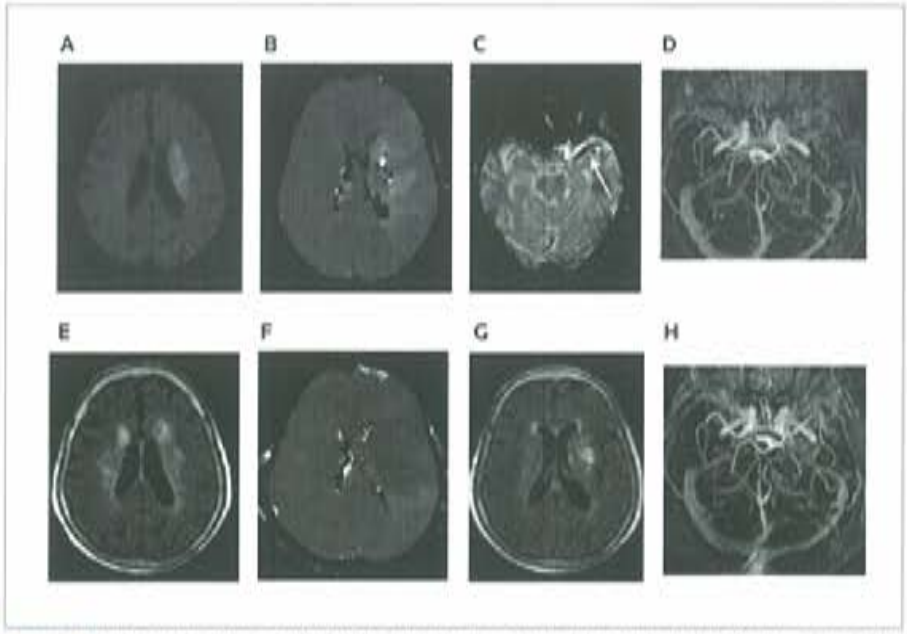

Figure 1. Brain images from a patient with acute stroke 4 hours after onset and an NIHSS score of 20. A) Diffusion-weighted imaging (DWI), showing an acute ischemic lesion of the left lenticular nucleus (deep territory of the left middle cerebral artery, MCA). B) Time-to-peak (TTP) map, a perfusion-weighted imaging (PWI) technique, revealing a more extensive perfusion alteration encompassing most of the superficial and deep territory of the MCA. The mismatch between DWI and PWI reveals the presence of significant penumbra. C) T2* map revealing the presence of a thrombus in the proximal artery (arrow). D) Magnetic resonance angiography (MRA), demonstrating severe occlusion of the left MCA with a lack of flow. The presence of a proximal occlusion and a small infarct (DWI–MRA mismatch) is another method to determine the presence of penumbra. E) A Fluid Liquid Attenuation Inversion Recovery (FLAIR) map, which does not show the acute stroke but reveals the presence of extensive chronic white matter lesions. F) TTP map after 3 hours, revealing the incomplete reduction of PWI alteration (partial reperfusion). G) FLAIR map after 2 hours of treatment, showing a mild hemorrhagic transformation of type II intraparenchymal hemorrhage. H) MRA follow-up after 2 hours of treatment with recombinant tissue plasminogen activator, showing the recovery of normal flow in the left MCA (complete early recanalization).

the ability to predict disease progression or treatment. Biomarkers should be strong, fast and simple to use and be supported by economic analyses.[2,7,14] Diverse criteria have been proposed for the validation of imaging biomarkers of stroke,[9,10,15] and we have combined them into a set of seven criteria for the validation of MRI-BAS (Table I).

TYPES OF IMAGING BIOMARKERS

Many MRI parameters are potentially good MRI-BAS. We selected the eight MRI-BAS most cited in the literature (Table II). Briefly, these MRI-BAS are defined as follows:

A. Symptomatic vessel patency. While vascular status has been classified in different ways, most studies determined initial vessel patency, final vessel status, early (< 6 hours after treatment) and late (3–5 days after treatment) recanalization.[16-20]

B. Infarct lesion volume. Some studies exclude small infarcts (< 10 cm^3) from treatment, while other authors have proposed considering infarcts larger than 100 cm^3 as malignant infarcts. The final infarct size is usually determined after 30 days' evolution.[21] Lesion growth is the volume change between the initial DWI study and the final study at day 30 using the Fluid Liquid Attenuation Inversion Recovery (FLAIR) sequence.

C. Reversibility of acute ischemic lesion. DWI reversibility is the percentage of the acute DWI lesion that does not overlap with the final infarction.[22]

D. Perfusion alteration. PWI can measure perfusion with maps of mean transit time, time to peak or maximum time (t_{max}), among others.[23] Early reperfusion has been defined as a 30% reduction in the initial PWI volume 4–8 hours after treatment.[18,21] Late reperfusion is the grade of reperfusion after 3 days.[24]

E. Penumbra volume determined as diffusion–perfusion mismatch. The ischemic penumbra is the critically hypoperfused tissue that can be salvaged from infarction by early reperfusion after acute ischemia.[25-28] If early reperfusion or a successful neuroprotective intervention does not occur, the core of the infarct will expand and the penumbra will be incorporated into the final infarct volume. The leading approach to the challenge of estimating the ischemic penumbra is based on the difference between the volume of tissue that exhibits a disturbance in cerebral blood flow as assessed by PWI, and the volume of tissue that has already developed evidence of advanced ischemic injury reflected by cytotoxic edema on DWI.[29-32] Most authors consider a significant penumbra to be present when the size of the PWI

Table I. Set of criteria for a valid magnetic resonance imaging (MRI) biomarker of stroke

1. The biomarker should be a biological, physiological, biochemical or anatomical change detectable with MRI.
2. The biomarker should be closely linked with the target of the disease treatment.
3. The biomarker should have a logical relationship with the severity of the disease. It is important to have a strong link to the true endpoint.
4. The detection and/or quantitative measurement of the biomarker should be accurate, reproducible and feasible.
5. New treatments (drugs or devices) can change the biomarker's value. The measured changes over time are closely linked to the success or failure of the therapy and to the true endpoint of the medical therapy being evaluated.
6. The biomarker can provide insight into the toxicity of a treatment.
7. Some MRI biomarkers are supported by a large body of scientific evidence and are thus highly recommended for clinical use in acute stroke. Preclinical assessment is valuable.

Table II. *Types of magnetic resonance imaging biomarkers of acute stroke*

A. Symptomatic vessel patency Initial vessel patency, final vessel status, early recanalization, late recanalization.
B. Infarct lesion volume Initial infarct volume, final lesion volume, lesion enlargement between days 1 and 3, lesion enlargement between days 1 and 30.
C. Reversibility of acute ischemic lesion Diffusion-weighted imaging reversibility.
D. Perfusion alteration Initial perfusion alteration, final perfusion alteration, early reperfusion, late reperfusion.
E. Penumbra volume determined as diffusion–perfusion mismatch
F. Penumbra volume determined as clinical–diffusion mismatch
G. Penumbra volume determined as diffusion–angiography mismatch
H. Hemorrhagic transformation of acute infarct Grades of hemorrhagic transformation.

lesion is > 120% of that of the DWI lesion [18,19] and other studies consider a minimum lesion volume of 10 cm^3 indicative of significant penumbra.[33] The concept of penumbra salvage is defined as the difference between the baseline PWI lesion and the final infarct volume.

F. Penumbra volume determined as clinical–diffusion mismatch. According to this model, the National Institute of Health Stroke Scale (NIHSS) score substitutes PWI lesion volume because PWI is difficult to assess in the acute setting in most centers. Clinical–diffusion mismatch (CDM) is defined as an NIHSS score ≥ 8 and a DWI lesion volume of < 25 mL.[34] Other authors have proposed the criteria of an NIHSS score ≥ 8 and a lesion volume of < 15 mL.[35]

G. Penumbra volume determined as diffusion–angiography mismatch. The following definition of DWI–MRA mismatch has been proposed: 1) DWI lesion volume of < 25 mL in patients with a proximal vessel occlusion; or 2) DWI lesion volume of < 15 mL in patients with proximal vessel stenosis or an abnormal finding in a distal vessel.[36]

H. Hemorrhagic transformation of acute infarct. Low-grade hemorrhagic transformation is a physiological process related to stroke recanalization and reperfusion. There are different grades of hemorrhagic transformation [37], but it is classified as significant (sICH) when it is associated with a worsening of at least four points on the NIHSS and when there is a dense hematoma (> 30% of the infarcted area) with a substantial space-occupying effect.[38]

CLINICAL AND THERAPEUTIC UTILITY OF MRI-BAS

Table III summarizes the correlations between each MRI biomarker and clinical outcome and their potential value in clinical trials.

A. Symptomatic vessel patency. Vascular occlusion by a thrombus is a clear treatment target. Recanalization has been correlated with infarct growth, DWI–PWI mismatch and favorable clinical response.[39]

B. Infarct lesion volume. Patients with large baseline DWI lesion volumes who achieve early reperfusion appear to be at greatest risk of sICH after rt-PA therapy [40] and the accepted criterion is to exclude lesions larger than one-third of the territory of the middle cerebral artery.[18] Infarct growth is the most commonly used imaging surrogate endpoint.[18,21,24,28,41,42]

C. Reversibility of acute ischemic lesion. DWI reversibility is related to early recanalization [43,44] and favorable clinical response.[22] This is a promising new biomarker. Future trials can determine its accuracy and reproducibility.[45]

D. Perfusion alteration. Early reperfusion has been a secondary [18] or primary [20] endpoint in some clinical trials. Early reperfusion correlates favorably with clinical outcome. Late reperfusion is associated with reduced infarct growth and improved neurological and functional outcomes.[24]

E. Penumbra volume determined as diffusion–perfusion mismatch. Patients with a DWI–PWI mismatch who have been treated with rt-PA more often demonstrate recanalization, reperfusion, reduced infarct growth and favorable clinical response than those without.[21,24,39] A DWI–PWI mismatch has been used to select patients for trials of intravenous thrombolysis with desmoteplase [18,19] and intra-arterial thrombolysis with alteplase or urokinase.[46]

F. Penumbra volume determined as clinical–diffusion mismatch. Patients with a CDM after treatment, especially those with a DWI–PWI mismatch, show less infarct growth compared with those without a CDM.[34,35]

G. Penumbra volume determined as diffusion–angiography mismatch. Reperfusion is associated with an increased rate of favorable clinical response in patients with a DWI–MRA mismatch.[36]

H. Hemorrhagic transformation of acute infarct. Hemorrhage is the main exclusion criterion for thrombolysis and sICH is associated with a poor outcome.[18,38]

CRITICAL ANALYSIS OF VALIDITY OF MRI STROKE BIOMARKERS

The application of the proposed validation criteria shows the current limitations of MRI-BAS.

1st criterion. Most MRI biomarkers are only structural and further studies should validate more biological, physiological and biochemical MRI-BAS. In this sense, future advances in MRI will allow the development of new imaging biomarkers related to the presence of inflammation, endothelial damage or edema.

2nd criterion. Few imaging biomarkers are closely linked to the presence of the treatment target. Vascular status is clearly related to the thrombus occluding the vessel, but further studies should redefine this parameter considering other variables such as the collateral circulation.

The detection of the penumbra is controversial and PWI abnormalities often overestimate critical hypoperfusion.[47-49] Therefore, the DWI–PWI mismatch concept needs to be refined.[29,50-52]

Table III. Validity of each MRI-BAS

MRI change	Vessel patency +++	Infarct lesion volume +++	DWI reversal +++	Perfusion alteration +++	PWI–PWI mismatch +++	CDM +++	DWI–MRA mismatch +++	Hemorrhagic transformation +++
Target	+++			++	++	++	++	
Correlation with severity of disease	+	+	+	+	+	+	+	+
Quality quantitative measurement	+	+	+	+	+	+	+	+
Treatments change value of MRI-BAS	+++	++	++	+++	+++	+++	+++	++
Inclusion criteria	+				+++		+	
Exclusion criteria	+++	+			+++			+++
Secondary endpoint	+++	+++		+++				++
Toxicity assessment								+++
Evidence in publications	+	+	+	+	+	+	+	+

MRI-BAS, magnetic resonance imaging biomarkers of acute ischemic stroke; DWI, diffusion-weighted imaging; PWI, perfusion-weighted imaging; CDM, clinical–diffusion mismatch; MRA, magnetic resonance angiography.
+++ High correlation, ++ moderate correlation, + mild correlation.

3rd criterion. The real clinical correlation of some MRI biomarkers, such as a CDM [33] or a DWI–PWI mismatch, is controversial.[53-55] Further studies should redefine the value of these biomarkers and probably develop an imaging scoring system that integrates different MRI variables to predict outcome and monitor the efficacy of stroke treatment.[12]

4th criterion. Measuring the accuracy of MRI-BAS is hindered by the lack of standardization of MRI techniques between different vendors and centers.[23] The feasibility of MRI-BAS is limited and needs to be improved through the development of automatic systems to quantify lesion volume, vessel flow, penumbra and hemorrhagic transformation.

5th criterion. Stroke treatments can change the value of the MRI biomarkers, such as the vascular status or a DWI–PWI mismatch, and this change is related to the success of the therapy. However, to date, the usefulness of MRI in stroke trials is based mainly on local experience, expert opinions and published open-label studies. The lack of placebo-controlled trials using MRI [18-21] is a great impediment because it is not possible to separate the effects of spontaneous versus "rt-PA-induced" recanalization. Thus, it is essential to perform larger, randomized studies including MRI-BAS, such as DWI–MRA or DWI–PWI mismatches.[56]

6th criterion. Few biomarkers detect the toxicity or adverse effects of treatment, and we need to develop more imaging biomarkers to detect the direct toxicity of rt-PA and future therapies. Although hemorrhagic transformation is a good marker of an adverse effect of treatment, there is a need for an sICH predictor that incorporates multiple factors,[40,57-61] and further studies should analyze the promising value of the apparent diffusion coefficient.

7th criterion. Most publications about MRI-BAS only present type III evidence. Publications with type I and type II evidence are required, which reflect the results of well-controlled prospective, randomized, double-blind clinical trials.[62]

CONCLUSION

We propose a set of criteria for the validation of MRI-BAS. However, few current MRI-BAS fully meet these requirements. Further large, randomized trials will enable us to overcome the limitations of current MRI-BAS and to validate new biomarkers.

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DISCLOSURE

The authors declare no conflicts of interest.

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4.2. Artículo 2. *Investigación sobre el biomarcador volumen del infarto cerebral*

Reliability of the ABC/2 Method in Determining Acute Infarct Volume. J Neuroimaging. 2012 Apr; 22 (2): 155-9.

Clinical Investigative Study

Reliability of the ABC/2 Method in Determining Acute Infarct Volume

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ABSTRACT

BACKGROUND AND PURPOSE

Infarct volume is used as a surrogate outcome measure in clinical trials of therapies for acute ischemic stroke. ABC/2 is a fast volumetric method, but its accuracy remains to be determined. We aimed to study the accuracy and reproducibility of ABC/2 in determining acute infarct volume with diffusion-weighted imaging.

METHODS

We studied 86 consecutive patients with acute ischemic stroke. Three blinded observers determined volume with the ABC/2 method, and the results were compared with those of the manual planimetric method.

RESULTS

The ABC/2 technique overestimated infarct volume by a median false increase (variable ABC/2 volume minus planimetric volume) of 7.33 cm^3 (1.29, 22.170, representing a 162.56% increase over the value of the gold standard (variable ABC/2 volume over planimetric volume) (121.70, 248.52). In each method, the interrater reliability was excellent: the intraclass correlations were .992 and .985 for the ABC/2 technique and planimetric method, respectively.

CONCLUSIONS

ABC/2 is volumetric method with clinical value but it consistently overestimates the real infarct volume.

Keywords: Stroke, volume, infarct.

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Introduction

Ischemic stroke results in a high rate of disability.[1] Acute cerebral infarct volume is an important imaging biomarker:[2-5] larger infarcts are associated to an important mass effect, worse outcome, and greater risk of hemorrhagic transformation after thrombolytic treatment.[6,7] In this sense, infarcts occupying more than one-third of the territory of the middle cerebral artery (MCA) or measuring more than 100 cc have been proposed as exclusion criteria for thrombolytic treatment,[8] and infarct enlargement is often used as a surrogate endpoint for treatment failure.[8-13]

However, acute infarct volume assessment is subjective. The gold standard is the planimetric technique in which infarct volume is calculated after manually segmenting the borders of the infarct in each slice.[3-6] However, this method is time consuming and therefore cannot be applied in acute settings to guide treatment decisions. A faster, accurate, and reproducible way to calculate infarct volume in acute settings is needed, and the ABC/2 has been proposed to meet this need.[14] The ABC/2 is a fast volumetric method based on the formula of the ellipse;[15] it has been validated for hematoma management[15] but not for acute ischemic stroke.[16,17] Some publications[14,18] discuss the validity of ABC/2 for assessing acute infarct volume with respect to other methods.

We aimed to define the accuracy of ABC/2 in determining acute infarct volume with diffusion-weighted imaging (DWI) compared with the planimetric technique and to determine the interobserver reproducibility of ABC/2.

Material and Methods

This is a secondary study of consecutive patients with acute hemispheric ischemic stroke admitted within the first 12 hours after the onset of symptoms. The primary aim of this ongoing prospective study is to investigate whether the molecular factors associated with early neurological deterioration correlate to the evolution of the magnetic resonance imaging (MRI) abnormalities in the acute phase of territorial infarctions.

Inclusion Criteria

Inclusion criteria for this study were age older than 18, time from the onset of symptoms to inclusion <12 hours, and previous modified Rankin score <2. For the purpose of this investigation, only patients with an acute MCA infarction were studied. Exclusion criteria were coma on admission, pure lacunar syndromes, transient ischemic attack, previous cerebral infarction impeding the clinical and neuroradiological evaluation, severe chronic diseases, and inflammatory diseases.

The ethics committee approved the study and we obtained written informed consent from all patients or their relatives before imaging.

MRI Protocol

All patients underwent MRI on admission within the first 12 hours after stroke onset. Magnetic resonance (MR) images were obtained on a 1.5 T system (Gyroscan NT, Philips Medical Systems, Best, the Netherlands) with echoplanar capabilities of 22 mT/m gradient strength and maximum slew rate of 120 mT/m/ms. The MRI protocol included a DWI sequence that was obtained with a single-shot spin-echo echo-planar pulse with a diffusion gradient b value of 0 and 1,000 s/mm^2 along three axes. The other parameters were 20 slices, 6-mm slice thickness, 0 gap, 102 ms TE, 6,000 ms TR, 67 (epi factor), and 28 seconds' duration.

Volumetric Assessment

We measured infarct volume on the initial DWI sequence using two methods: the manual planimetric segmentation method and the ABC/2 method.

In the manual planimetric method, we traced the perimeter of the area of abnormal signal intensity on each DWI map and then used Philips workstation volumetric software to estimate the total volume based on the area traced and thickness of each slice. The window level and window width were chosen to obtain the best contrast between the lesion and the surrounding normal tissue.

In the ABC/2 method (Fig 1), we selected the slice where the infarct looked largest, measured the largest diameter of the infarct in this slice (A) and its largest perpendicular diameter (B), and then determined the vertical diameter (C) by summing the thickness of the slices in which the lesion was visible. Finally, we calculated infarct volume using the formula $.5 \times A \times B \times C$.[7,19]

In some patients, the infarct appeared as a combination of multiple scattered small lesions. In these cases, the volume of each lesion was determined and the global volume was obtained with the sum of the different volumes.

Three observers with experience in acute stroke imaging who were blinded to the clinical characteristics and stroke outcome analyzed the volume on the initial DWI sequence. Each observer calculated the volume three times with the ABC/2 method; observers were blinded to their previous measurements. The mean value was used for all analyses. To avoid a learning effect during the study, the measurements were performed after a period of 6 months' experience in the volumetric assessment of ischemic lesions. For each method, we determined intraobserver variability by comparing the data from each observer at two different measuring sessions, and we determined interobserver variability by comparing the three observers' first measurements. The time between the first and second assessments of a scan with the same method was at least 1 week.

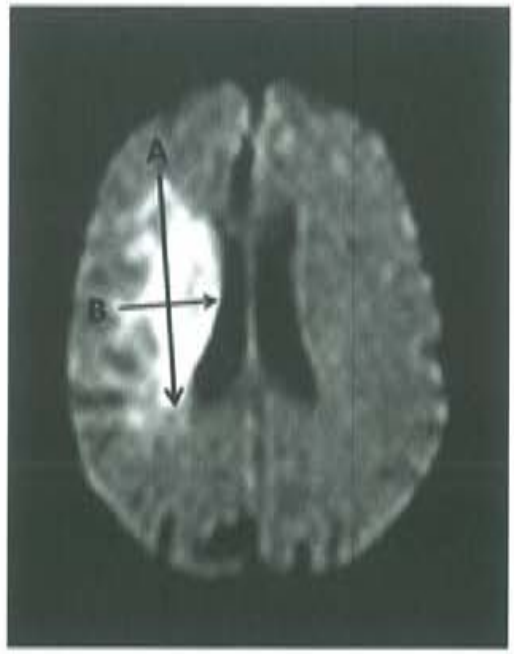

Fig 1. ABC/2 method. First, the largest diameter (A) of the infarct and its largest perpendicular diameter (B) were measured with a caliper. Then, the vertical diameter (C) was determined by summing the thickness of the slices in which the lesion was visible. Infarct volume was calculated according to the formula $.5 \times A \times B \times C$.

MCA infarcts were divided into three groups on the basis of the territory involved as follows: (1) deep (caudate, lenticular nuclei, and/or internal capsule), (2) superficial (cortical structures, including the insula), or (3) both territories.

Statistical Analysis

Analyses were performed with R (R Foundation for Statistical Computing, Vienna, Austria) and Minitab 15.1 software (State College, PA). Categorical variables are presented in percentages. Continuous variables are presented as mean ± standard deviation (SD) or median (quartiles), depending on their distributions. Continuous variables were compared between two groups with Student's t-test or the Mann-Whitney test as appropriate. Proportions were compared with the χ^2 test. Statistical significance was set at $P < .05$. We analyzed the variability of the measurements by Bland and Altman plots showing the difference between three measurements against their mean and by calculating the intraclass correlation coefficient for each method.

Results

Over a 3-year period, 91 patients (mean age, 69.68 ± 11.26 years; range, 40-90 years) were included in the study. Five

Table 1. Infarct Volume by Observer in the ABC/2 and Planimetric Methods

Method	Observer	Mean ± SD	Q1	M	Q3
ABC/2	1	39.20 ± 53.67	8.40	17.57	49.23
	2	45.60 ± 60.09	8.07	21.87	55.71
	3	42.53 ± 56.57	8.13	18.50	54.02
Planimetric	1	24.30 ± 34.81	5.21	10.36	22.83
	2	21.74 ± 29.51	4.22	9.84	25.46
	3	23.46 ± 32.56	4.95	10.02	24.52

All values are in cc. Mean ± SD = mean volume and standard deviation, Q1 = 1st quartile, M = median, Q3 = 3rd quartile.

patients were excluded because of motion artifacts; thus, we studied 86 stroke patients (mean age, 69.37 ± 11.4; range, 40-90 years). All patients were studied in the first 12 hours after stroke onset (mean, 2.8 hours; range, .25-9.8 hours).

All patients had MCA infarction: 52 in the superficial territory of the MCA (60.5%), 19 in the deep territory (22.1%), and 15 in both territories (17.4%).

Infarct volumes measured with the ABC/2 method (19.34 cc [8.20, 52.94]) (median, 1st Q, 3rd Q) were significantly higher than those measured with the planimetric method (9.93 cc [4.92, 23.09]) ($P < .0001$). Table 1 shows the differences between the two methods for each observer.

With respect to the gold standard, the ABC/2 technique overestimated infarct volume by a median false increase (variable ABC/2 volume minus planimetric volume) of 7.33 cm^3 91.29, 22.170, representing a 162.56% increase over the value of the gold standard value (variable ABC/2 volume over planimetric volume) 9121.70, 248.52).

Figure 2 shows the correlation between the two methods and the slope of the graph using fitted line plots in observer 1, observer 2, and observer 3, respectively.

Table 2 shows the infarct volume with respect to the territories affected by the infarct (deep, superficial, or both territories). In all three types, the slope was significantly greater than 1 ($P < .001$ for superficial or deep infarcts, and $P < .0273$ for infarcts affecting both territories). This analysis supports the overestimation of the ABC/2 method with respect to the planimetric method.

The interobserver reliability was excellent for both techniques: the intraclass correlation coefficient was .992 for the ABC/2 technique and .985 for the planimetric method. All three observers chose the same slice in the DWI sequences for the ABC/2 technique in 65.1% of cases.

The intraobserver variability of the ABC/2 method in observer 2 was studied with different methods and yielded the following results: (1) the Pearson correlation was .998, (2) the significance level of the Mann-Whitney test was .9841, and (3) the Bland-Altman plot confirmed the intraobserver reliability (Fig 3).

Discussion

In our study, three results support the conclusion that ABC/2 is not an accurate method for the measurement of ischemic lesion volume. First, the ABC/2 technique overestimated infarct volume by a median false increase (variable ABC/2 volume minus planimetric volume) of 7.33 cm^3 (1.29, 22.17), a 162.56% increase (variable ABC/2 volume over planimetric volume) over the gold standard value (121.70, 248.52). Second, the overestimation was confirmed in the three observers. Third, the ABC/2 method overestimated infarct volume regardless of the vascular territory affected.

Three results support the conclusion that the reproducibility of ABC/2 is adequate: first, the intraclass correlation was .992; second, the three observers chose the same slice for ABC/2 measurement in 65.1%; and third, the intraobserver reliability was very high.

The ABC/2 technique has been used in some stroke papers,[16,17] but few studies have examined its validity. Van der Worp et al.[4] studied the inter and intra-observer variability of different volumetric methods and found higher reproducibility for the planimetric method than for ABC/2 or other approaches. However, these authors did not compare the accuracy of ABC/2 with respect to the planimetric method. In this sense, our analysis reveals that the ABC/2 method significantly overestimates the infarct volume determined with the planimetric method.

Sims et al.[14] recently reported that the ABC/2 ellipsoid formula was more accurate than spherical and cylindrical approaches for estimating infarct volume and that the intra and inter-observer reliability of ABC/2 was high for both acute and subacute infarcts; they concluded that the ABC/2 method is much better than the other fast methods for determining acute stroke treatment. However, our study suggests that the accuracy of the ABC/2 method is limited because it overestimates the infarct volume determined with the gold standard method.

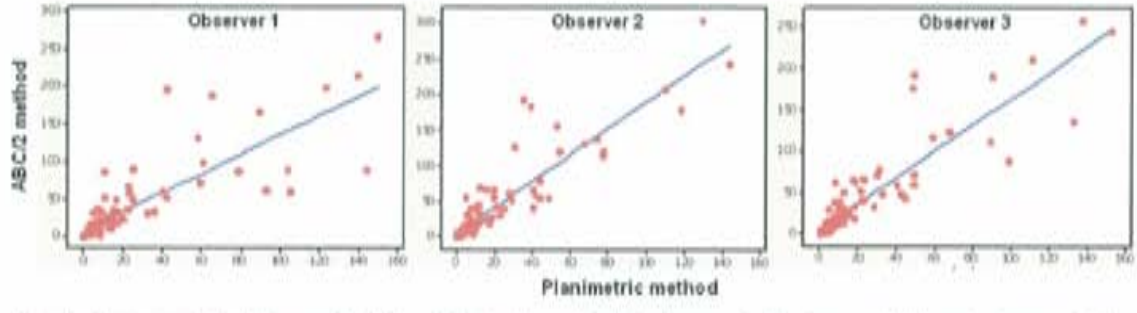

Fig 2. Infarct volume correlation between ABC/2 and planimetric methods in observer 1 (A), observer 2 (B), and observer 3 (C).

Table 2. Infarct Volume by MCA Territory in the ABC/2 and Planimetric Methods

Type	ABC/2	Planimetric	Correlation	Slope	R^2
Superficial	9.04 (2.00, 15.98)	5.350 (1.88, 9.21)	.83	1.62	69.4%
Deep	21.38 (8.92, 50.64)	9.84 (5.13, 22.49)	.86	1.62	75.4%
Superficial + deep	61.2 (23.4, 130.8)	40.09 (14.93, 72.810	.79	1.29	63.0%
Global	19.34 (8.20, 52.94)	9.93 (4.92, 23.09)	.86	1.52	75.4%

All values are in cc. Median, Q1 = 1st quartile, Q3 = 3rd quartile.

One explanation for this overestimation might be related to the irregular shape (not similar to an ellipse) of most infarcts (Fig 1). Thus, investigators using ABC/2 to determine infarct volume in the acute setting should know this limitation.

Our results support the use planimetric method because it is more accurate. However, this method is time consuming, it has a significant learning curve, and it is not feasible in the acute setting. In this scenario, the ABC/2 method can be used to obtain a fast assessment of volume of the infarct provided its advantages and limitations are clearly understood.

A faster, reliable automatic planimetric volumetric method needs to be developed to determine the volume of the ischemic lesion and the volume of the mismatch between diffusion and perfusion alterations in the acute setting and in the FLAIR sequence after 30 days' evolution.

Acute infarct volume could be considered an imaging biomarker [3,20-22] because it can be used to determine whether to include patients in new treatment trials and to predict the outcome of stroke patients. A recent paper[3] established the criteria for valid MRI biomarkers for acute ischemic stroke. One criterion is an accurate, feasible, reproducible technique for measuring infarct volume. The variable accuracy of volumetric methods is also related to the lack of standardization of MRI techniques between different vendors and centers.[23] The development of automatic systems for quantifying lesion volume can make infarct volume assessment more feasible. Different commercial and academic informatics tools to determine infarct volume can automatically quantify the volume of ischemic lesions and integrate this information in the electronic medical records,[24] studies should be undertaken to validate these systems.[25]

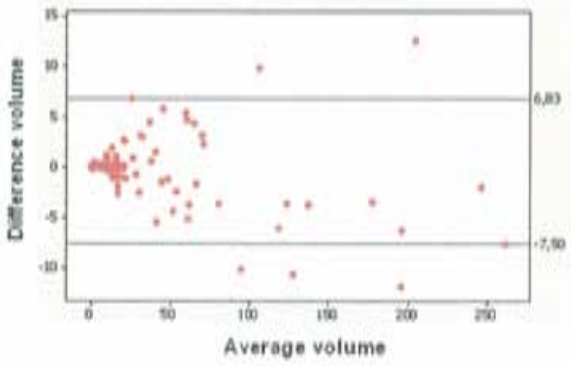

Fig 3. Bland-Altman plot confirmed the intraobserver reliability. The figure shows that most values are within the confidence limits.

Our study has some limitations that merit comment. First, we studied a group of infarcts with small volumes (19.34 cc [8.20, 52.94]). Further studies should confirm the findings in a sample with larger infarcts.

Second, we did not analyze the validity of the ABC/2 method in the measurement of the mismatch volume or in the assessment of final infarct volume. Further studies should address the value of ABC/2 in these scenarios with a large sample.

Third, we did not analyze the therapeutic value of ABC/2 method. Some previous papers have shown the clinical value of infarct volume determined with the ABC/2 method in clinical decision making in acute settings.[16-18] These authors suggested that errors in estimating the volume of small infarcts have little impact on clinical decision. Further studies should analyze the final therapeutic utility of new volumetric techniques with respect to ABC/2 method.

Fourth, we included only MCA infarcts and we excluded lacunar syndromes. Volume could be an important prognostic factor in any type of infarct; however, we decided to study MCA infarcts because most trials investigating new treatments concentrate only on supratentorial infarcts, and these correspond mainly to MCA infarcts. In this sense, our priority to validate infarct volume as an imaging biomarker for the treatment of MCA infarcts.[3] Further studies should address the validation of volume in all kinds of infarcts.

Conclusion

Our findings indicate that the ABC/2 method consistently overestimates the volume of infarction compared with the planimetric method. Although ABC/2 is less accurate, it is reproducible and fast and may be useful in the acute setting provided its limitations are taken into consideration.

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4.3. Artículo 3. *Investigación sobre el biomarcador topografía de lesión de tractografía de TCE*

Acute Damage to the Posterior Limb of the Internal Capsule on Diffusion Tensor Tractography as an Early Imaging Predictor of Motor Outcome after Stroke. AJNR Am J Neuroradiol. 2011 May; 32 (5):857-63.

ORIGINAL RESEARCH

Acute Damage to the Posterior Limb of the Internal Capsule on Diffusion Tensor Tractography as an Early Imaging Predictor of Motor Outcome after Stroke

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BACKGROUND AND PURPOSE: Early prediction of motor outcome is of interest in stroke management. We aimed to determine whether lesion location at DTT is predictive of motor outcome after acute stroke and whether this information improves the predictive accuracy of the clinical scores.

MATERIALS AND METHODS: We evaluated 60 consecutive patients within 12 hours of middle cerebral artery stroke onset. We used DTT to evaluate CST involvement in the motor cortex and premotor cortex, centrum semiovale, corona radiata, and PLIC and in combinations of these regions at admission, at day 3, and at day 30. Severity of limb weakness was assessed by using the motor subindex scores of the National Institutes of Health Stroke Scale (5a, 5b, 6a, 6b). We calculated volumes of infarct and fractional anisotropy values in the CST of the pons.

RESULTS: Acute damage to the PLIC was the best predictor associated with poor motor outcome, axonal damage, and clinical severity at admission ($P < .001$). There was no significant correlation between acute infarct volume and motor outcome at day 90 ($P = .176$, $r = 0.465$). The sensitivity, specificity, and positive and negative predictive values of acute CST involvement at the level of the PLIC for motor outcome at day 90 were 73.7%, 100%, 100%, and 89.1%, respectively. In the acute stage, DTT predicted motor outcome at day 90 better than the clinical scores ($R^2 = 75.50$, $F = 80.09$, $P < .001$).

CONCLUSIONS: In the acute setting, DTT is promising for stroke mapping to predict motor outcome. Acute CST damage at the level of the PLIC is a significant predictor of unfavorable motor outcome.

ABBREVIATIONS: BI = Barthel index; CR = corona radiate; CS = centrum semiovale; CST = corticospinal tract; DTI = diffusion tensor imaging; DTT = diffusion tensor tractography; DWI = diffusion-weighted MR imaging; FA = fractional anisotropy; IV = intravenous; MC = motor cortex; MCA = middle cerebral artery; m-NIHSS = motor subindex scores of the National Institutes of Health Stroke Scale; MRI = MR imaging; mRS = modified Rankin scale; NS = not significant; PLIC = posterior limb of the internal capsule; PMC = premotor cortex; PWI = perfusion-weighted imaging; rFA = FA ratio; ROI = region of interest; r_{pb} = point-biserial correlation coefficient; rtPA = recombinant tissue plasminogen activator; WD = Wallerian degeneration

Accurate early prediction of motor functional outcome in the early stage of stroke is important for clinicians and researchers in management and rehabilitation.[1] Motor deficit after stroke is common and has a considerable influence on quality of life.[2] Several observational studies have demonstrated that the grade of initial motor deficit is the most important determinant of motor recovery.[1-5] Other valid predictors in regression models have included infarct site, volume of stroke, age, demographics, comorbidities, infarct side, and stroke subtype.[1-3,6]

The CST is the main pathway that mediates voluntary movements, and neurophysiological and structural imaging studies have evidenced that motor outcome is heavily dependent on the integrity of the motor fibers.[6-13] Thus, the involvement of motor-related cortical regions, CR, and internal capsule progressively decrease the probability of upper limb functional recovery.[6,14] Recently, these findings were complemented by DTI studies that have demonstrated the usefulness of DTT for predicting poor motor outcome when infarct involves the CST.[15-20] DTI enables in vivo visualization and quantification of microstructural damage to white matter tracts.[21] DTT uses data acquired through DTI to reconstruct a 3D macroscopic orientation of the white matter fibers that enables the specific topographic relation between lesion location and CST fibers to be evaluated.[22] Decreases in FA, a DTI-derived structural measure, have been interpreted as WD and

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proposed as an index of axonal damage.[23] Decreased FA in the CST correlates with motor impairment 1 month after stroke.[24] In contrast, although patients with large infarcts tend to have a poor outcome, functional deficits due to moderate-sized infarcts are more difficult to predict.[7,25,26] One of the major reasons functional outcome does not correlate strongly with infarct volume is that the specific site of the lesion is not taken into account.[4,9]

In the current study, we aimed to 1) evaluate whether the specific site of a lesion in the CST (primary MC, PMC, CS, CR, PLIC, and combinations among these) at DTT predicts axonal damage to the motor pathway and functional motor outcome after acute stroke, and 2) assess whether a model incorporating DTT information on the specific location of the stroke and clinical scores is more accurate in predicting motor outcome than clinical scores alone.

Materials and Methods

Patients

The data reported here were obtained from the same cohort of patients included in our previous study relating WD and motor outcome.[24] Patients included had a nonlacunar, first-ever MCA infarction and were admitted to our stroke unit within 12 hours of symptom onset during a 19-month period. Patients with other lesions, cerebral hemorrhage, significant pre-existing nonischemic neurologic deficit (including dementia or extrapyramidal disease), or a history of prior stroke that would hinder the interpretation of clinical and imaging data were excluded. Our institutional ethics committee approved the study, and written informed consent was obtained from all patients or from close relatives.

Clinical Examination

A senior certified staff neurologist used the National Institutes of Health Stroke Scale to assess clinical deficit at admission, at day 3, at day 30, and at day 90 from stroke onset. The m-NIHSS subindex (5a, 5b, 6a, 6b) was used to categorize the severity of limb weakness as grade I (total m-NIHSS score of 0), grade II (m-NIHSS, score of 1–4), or grade III (m-NIHSS, score of 5–8). The mRS and BI were used to measure disability and dependence in activities of daily living at day 90. Poor overall outcome was defined as mRS >3, BI <60, or both.[27] All clinical assessments were performed without knowledge of the MR imaging findings. Patients were treated according to published guidelines.[28]

MR Imaging Protocol

All scans were performed with a whole-body 1.5T MR system (Gyroscan Intera; Philips Medical Systems, Best, the Netherlands) with a SENSE head coil. The routine protocol included axial trace DWI, fluid-attenuated inversion recovery, T2-weighted gradient-echo, perfusion-weighted imaging, time-of-flight angiography, and DTI sequences. DTI was performed by using a single-shot echo-planar imaging sequence with the sensitivity encoding parallel-imaging scheme (acceleration factor, 2) after contrast agent administration. Diffusion-sensitized gradients were applied along 15 noncollinear directions with a b-value of 1000 s/mm^2. In addition, diffusion-weighted B0 images were obtained. Other acquisition parameters were TR/TE, 6795 ms/72 ms; 23 × 23-cm FOV; and 112 × 112 matrix size. DTI voxel size was 2.05 × 2.05 × 3 mm. Forty sections covering the entire brain were obtained parallel to the bicommissural line without intersection gaps. DTI acquisition took 3 minutes and 10 seconds.

Data Processing and DTI

Diffusion-sensitized image sets were transferred to an off-line workstation for data analysis. We used DTIWeb version 2.0 (http://trueta.udg.edu/DTI/index.html) to calculate tensor values for tractography.[29] Anisotropy maps were obtained by using orientation-independent FA, and color FA maps were generated following the standard convention (red, left-right; green, anteroposterior; and blue, superior-inferior).

Tractography was based on a diffusion tensor deflection algorithm.[30] The threshold for stopping fiber propagation was FA <0.2 and angle <70°. The seeding method put 1 starting seed randomly inside each voxel with an FA >0.4. To reconstruct the CST, the ROIs were placed at the level of the cerebral peduncle and around the CR in the direction-coded color axial sections. Unrelated fibers, such as those going to the contralateral hemisphere, cerebellum, or thalamus, were removed by using specific ROIs. All ROIs were placed by 2 of the authors (A.P.-G., G.B.); the CST depicted and the evaluation of the PMC were validated by using landmarks from neuroanatomy atlases.[31]

Assessment of Damage to Specific CST Regions

To decide which structures were affected by infarct, the tractograms of CSTs were superimposed on DWI, and the following specific regions were evaluated: MC, PMC, CS, CR, PLIC, and combinations of these regions (On-line Fig. 1). These regions were scored separately on each section on 2 separate occasions 6 weeks apart by 1 rater (J.P.) and once by 2 raters (J.P., S.R.); all raters were blinded to the clinical ratings. Discordant ratings were resolved by consensus.

Measurement of the FA Values of CSTs

First, FA values for each region of interest on axial sections of the affected and unaffected CST at the rostral pons were obtained by averaging all voxels of 3 contiguous sections. Second, the ipsilateral-to-contralateral CST FA ratios were calculated ($rFA = FA_{affected\ side}/FA_{unaffected\ side}$). Two readers (J.P., G.B.) blinded to the clinical scores quantified FA.[24]

Calculation of Infarct Volume

Infarct volumes were determined off-line. Two readers (J.P., G.B.) manually outlined the areas of abnormal hyperintensity on each axial trace DWI. Surface areas of abnormal hyperintensity were summed and multiplied by section thickness (6 mm) and intersection gap (1 mm) to calculate infarct volumes. The results of the 2 readers were averaged.[24]

Statistical Analysis

To determine whether acute-stage involvement of specific CST regions and combinations of CST regions were associated with stroke severity, clinical and motor outcome at day 30 and at day 90, axonal damage, and/or acute-phase infarct volume, we used the chi-squared test to compare categoric variables and Student *t* test to compare quantitative variables. We used the Cohen κ coefficient to assess intraobserver and interobserver reliability. Intra- and interobserver agreement were classified as slight (κ = 0.0–0.20), fair (κ = 0.21–0.40), moderate (κ = 0.41–0.60), substantial (κ = 0.61–0.80), or almost perfect (κ = 0.81–1.00) according to the scale proposed by Landis and Koch.[32]

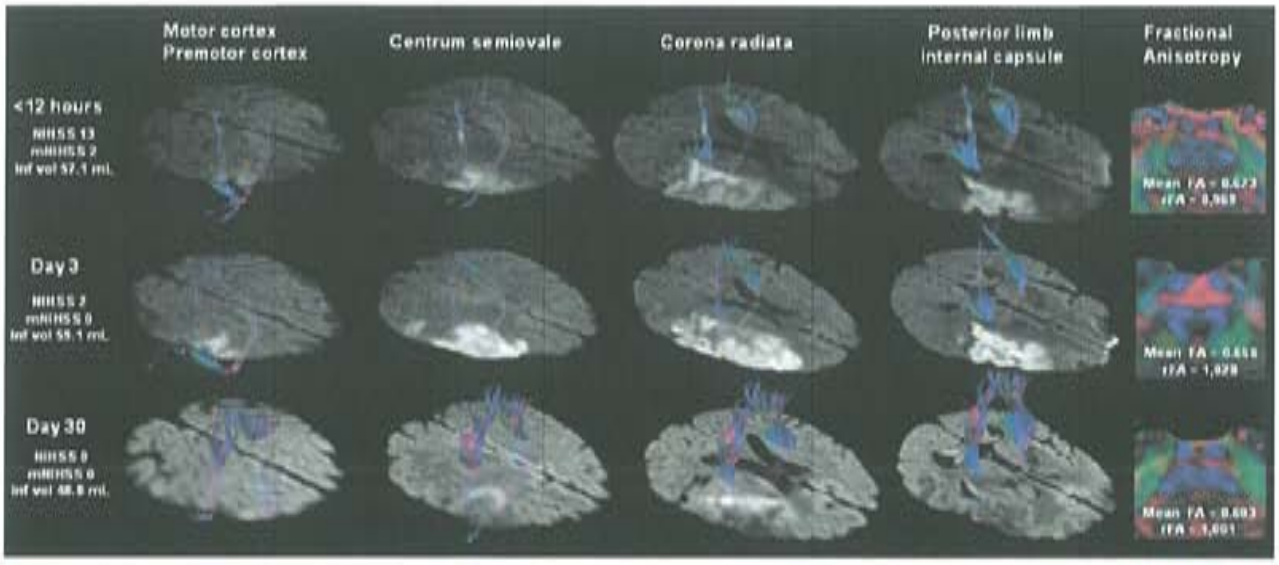

Fig 1. A 55-year-old man (patient 40) presented with moderate right peripheral MCA territory infarction. DTT images show infarction near the right CST at the level of the CR and PLIC, although there is no direct involvement. At admission, ischemic penumbra (not shown) involved part of the CST at the level of the CR and could explain the motor deficit at this time. FA indices reveal CST axonal integrity at the anterior part of the pons.

Motor outcome was first analyzed by using bivariate statistics. We calculated the correlation coefficients for lesion site and for clinical scores with motor deficit at day 3, day 30, and day 90. Each specific CST region was coded as 0 (unaffected by infarct) or 1 (affected by infarct). The predictive dataset contained both dichotomous (involvement of specific CST region) and numeric and ratio variables (infarct volume, rFA, and m-NIHSS) for which an r_{pb} or correlation coefficient was used, respectively. Coefficients with a P value $\leq .05$ were considered significant.

Multiple regression analysis was used to predict motor outcome at day 90 after stroke by using a combination of motor deficit, specific CST region involved, and imaging data. We also evaluated the additional predictive value conferred by adding the effect of region involved to that of the motor deficit. The dependent variable was the m-NIHSS score at day 90 after stroke, predicted from the following combinations of independent variables: 1) the specific CST region, m-NIHSS, and infarct volume in the first 12 hours after stroke; 2) the specific CST region, m-NIHSS, and infarct volume at day 3; and 3) the specific CST region, m-NIHSS, infarct volume, and rFA at day 30. To determine which combination of independent variables yielded the best predictive model, variables were deleted one by one from the model on the basis of the significance of their regression coefficients and the R^2 selection method. The models with the highest R^2 and all predictor variables that were significant ($P < .05$) were retained for each prediction. Only the model selected for the dataset obtained at day 3 fulfilled the assumption of normality. All statistical analyses were performed by using MINITAB version 15.1.0.0 (Minitab, State College, Pennsylvania).

Results

Subjects

Sixty-five consecutive patients with ischemic MCA stroke were scanned on admission, but data from 5 patients were incomplete at day 90 due to recurrence of stroke, death, and the presence of motion artifacts. Analyses were therefore based on 60 subjects (37 men, 23 women; aged 68 ± 13 years). One patient missed the MR imaging study at admission but completed studies on day 3 and day 30. All patients underwent MR imaging and clinical assessment at day 30.

Clinical Characteristics and DTT Analysis

On-line Table 1 presents detailed clinical and MR data for all the patients. The m-NIHSS score at admission was 11 (interquartile range 7–17), indicating that most patients had moderate to severe neurologic deficits. All patients had started physiotherapy within 2 weeks after the stroke. At admission, 47 (78.3%) of 60 patients presented some motor deficits, and 28 (59.6%) of these patients had moderate-severe motor deficit (m-NIHSS III). At day 3, 28 patients (46.7%) in total presented some motor deficits, and 13 (46.4%) of these patients were classified at m-NIHSS III. Improvements with respect to baseline scores were observed in 67.8% of patients at day 30 and in 85.7% at day 90, and 42.8% and 39.2% of motor deficits were categorized as m-NIHSS III at day 30 and day 90, respectively. The MCA territories most frequently involved were the peripheral territory, striatocapsular territory, or both. Thirty-one patients (51.67%) received intravenous rtPA (alteplase). There were no significant differences in rtPA treatment at admission among the m-NIHSS groups at day 90. BI and mRS scores before the stroke were 100 and 0 in all patients, respectively.

The mean time for reconstructing and assessing the DTT to evaluate the damage to CST regions was 3 minutes and 30 seconds. At admission, the CST did not seem disrupted or displaced in any patient. Intrarater and interrater agreement about the region of the CST affected was almost perfect (κ = 0.88 and κ = 0.84, respectively). No CST involvement by infarct on admission was observed in 14 (23.34%) patients; however, 5 of these had motor deficits (On-line Table 1). In contrast, CST involvement was observed on admission in 5 patients without motor deficits; the areas affected were the PMC (n = 2), PMC and CR (n = 1), CR (n = 1), and PLIC

Table 1: Sensitivity, specificity, and positive and negative predictive values for motor outcome according specific CST regions in acute stroke

	Motor Outcome	m-NIHSS	Sensitivity	Specificity	PPV	NPV
PLIC <12 hr	Day 30	I vs. II/III	66.67	100.00	100.00	84.78
		II vs. III	100.00	70.00	78.57	100.00
	Day 90*	I vs. II/III*	73.68*	100.00*	100.00*	89.13*
		II vs. III*	100.00*	71.43*	85.71*	100.00*
PLIC at day 3	Day 30	I vs. II/III	71.43	100.00	100.00	86.67
		II vs. III	100.00	60.00	73.33	100.00
	Day 90	I vs. II/III	78.95	100.00	100.00	91.11
		II vs. III	100.00	57.14	80.00	100.00
CS at day 3	Day 30	I vs. II/III	68.75	87.18	68.75	77.27
		II vs. III	54.55	90.00	54.55	90.00
	Day 90	I vs. II/III	47.37	82.93	56.25	77.27
		II vs. III	50.00	57.14	66.67	40.00
CR at day 3	Day 30	I vs. II/III	71.43	56.41	46.88	78.57
		II vs. III	63.64	20.00	46.67	33.33
	Day 90	I vs. II/III	73.68	56.10	43.75	82.14
		II vs. III	58.33	0.00	50.00	0.00

* Indicates the highest overall values for all determinations.

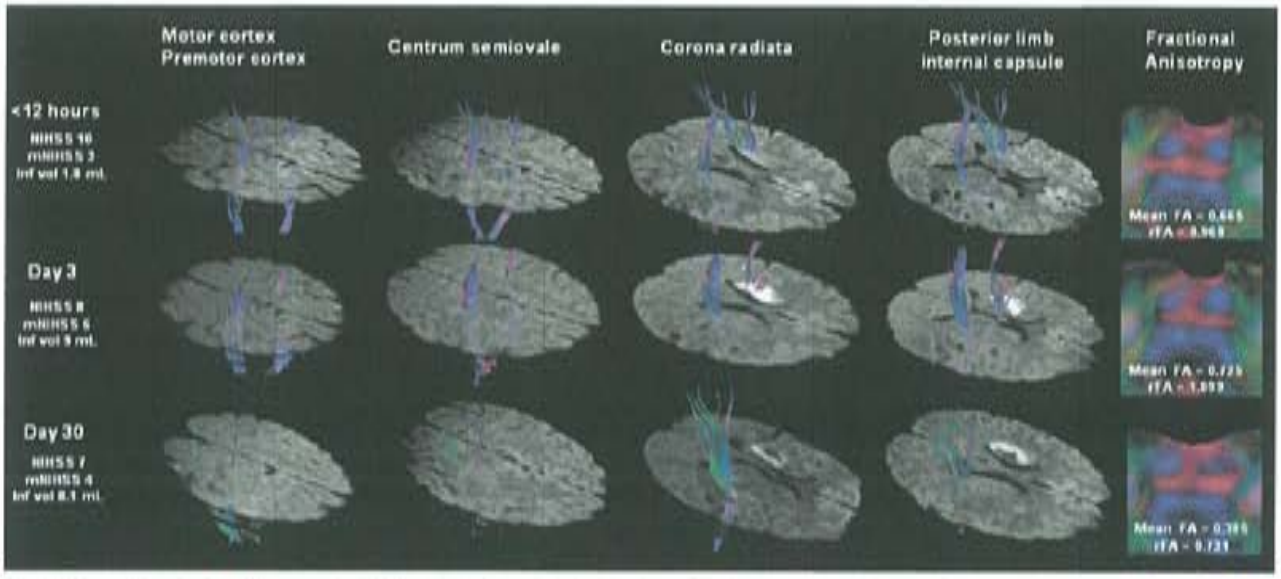

Fig 2. A 47-year-old man (patient 15) presented a mild right-sided hemiparesis lasting 45 minutes. DTT of motor tracts superimposed on DWI are shown tridimensionally. A small infarct in the striatocapsular MCA territory involves the left CST. Note the slight hyperintensity of the CST involved by the infarct due to the short time elapsed since the onset of symptoms. The markedly reduced brightness on the left side and decreased FA value in the left descending CST at day 30 are regarded as WD.

($n = 1$). At day 30, involvement of at least one specific CST region was observed in all patients with motor deficit. Finally, PLIC involvement in the first 12 hours was associated with unfavorable overall outcome (mRS >3, BI <60, or both) ($P < .001$).

Motor Outcome Prediction and the Involvement of the Specific CST Regions

Damage to the PLIC in the first 12 hours and at day 3 after stroke correlated with clinical severity, axonal damage expressed as decreased FA and rFA values, and motor outcome at day 30 and day 90 ($P < .001$) better than damage to any other CST region (Table 1). There was no significant correlation between acute infarct volume and motor outcome at day 90 ($P = 0.176$, $r = 0.485$) (Figs 1 and 2).

CS involvement, CR involvement, or both at day 3 was associated with motor deficit at day 30 and day 90 ($P < .004$) and axonal damage ($P < .003$) (On-line Table 2). It is noteworthy that although other significant associations can be observed, combined PLIC and CS or CR involvement at day 3 was not significant for motor outcome at day 30 or at day 90 ($P = .157$ and $P = .218$ for the interaction between PLIC and CS at day 30 and at day 90, respectively; $P = .521$ and $P = .457$ for the interaction between PLIC and CR at day 30 and at day 90, respectively). Therefore, the motor outcome at day 30 and at day 90 is secondary to PLIC damage.

Damage to the PLIC in the first 12 hours yielded the highest sensitivity, specificity, and predictive values for the prediction of motor outcome at day 90. Interestingly, PLIC damage by acute stroke clearly distinguishes subjects without motor deficit (m-NIHSS I) from those with motor deficit (NIHSS II and III) and even differentiates m-NIHSS II from m-NIHSS III at day 90 (Table 1).

Correlations analysis revealed significant coefficients only between PLIC involvement in the first 12 hours and motor outcome at day 90 (On-line Table 3). Damage to the CR, CS,

Table 2: Models selected from multiple regression analyses for predicting m-NIHSS 90 days after stroke from motor scores and specific CST regions

Predictor	Regression Coefficient	*t* Value	Added R^2
Measurements obtained <12 hr (R^2 = 75.50, F = 80.09**)			
PLIC damage	5.36	8.95**	75.50
Constant	0.64		
Measurements obtained at 72 hr (R^2 = 85.62, F = 74.39**)			
m-NIHSS	0.75	5.72**	79.00
PLIC damage	2.28	3.39*	6.62
Constant	−1.58		
Measurements obtained at day 30 (R^2 = 90.10, F = 236.72**)			
m-NIHSS	0.96	15.39**	90.10
Constant	−0.40		

* $P < .01$; ** $P < .001$.

or both; m-NIHSS; and acute-stage infarct volume were not related to motor outcome at 90 days. At day 3, PLIC damage and m-NHISS showed the most significant correlations with motor outcome at 90 days. At day 30, PLIC damage, m-NHISS, and axonal damage showed the most significant correlations with motor outcome at day 90. The only relation between infarct volume and motor outcome at day 90 was a modest correlation observed at day 3.

Table 2 summarizes the best predictive models achieved at each time point. The simplest model to predict m-NIHSS at day 90 based on the data available in the first 12 hours consisted only of PLIC damage; PLIC damage alone accounted for 75.5% of the variance in outcome. At day 3, regression analyses indicated that m-NIHSS accounted for 79% of the variance in motor outcome at day 90, and PLIC damage had a significant contribution of only 6.62%. Regression coefficients for these assessments were positive, indicating that an infarct affecting CST and m-NIHSS is predictive of greater motor deficit from day 3 to 90 days after stroke. The best model for predicting motor outcome at day 90 based on the assessments at day 30 included only the m-NIHSS, which accounted for 90.10% of the variance in the measurement.

Association between the Region of the CST Affected in the First 3 Days after Stroke and FA Indexes at Day 30

Our previous study demonstrated that mean FA values along the affected CST were significantly lower than the normal contralateral side only at day 30 after stroke onset ($P < .001$), and these values were lower than the corresponding FA values obtained at admission and at day 3. Moreover, the decrease in mean FA values correlated positively with the motor deficit at 30 days after stroke.[19] Combined involvement of the PLIC and CS or CR at day 30 was not significantly associated with decreased FA indexes ($P = 0.445$ for the interaction between PLIC and CS; $P = .830$ for PLIC and CR). Hence, axonal damage reflected as decreased FA ratio values at day 30 was also secondary to PLIC damage. There was no association between infarct volume and WD ($r = -0.221$, $P = .090$).

Discussion

We sought to determine whether acute stroke damage to specific CST regions evident at DTT can predict limb motor outcome on a categoric scale based on the m-NIHSS. We found that the involvement of the PLIC alone or in combination with other specific CST regions in the first 12 hours after stroke was strongly associated to severe motor deficits in the first 12 hours and poor motor functional outcome at day 90. Although damage to the CS and CR at day 3 was also associated with poor motor outcome at day 90, PLIC damage in the first 12 hours after stroke was clearly the best predictor of motor deficits and of their severity.

Predictors of motor outcome proposed include location and extension of the stroke specifically within the CST, grade of initial motor deficit, and infarct volume. Our findings corroborate previous studies that found motor outcome is strongly dependent on the integrity of the CST and that the involvement of regions such as the PLIC with more attenuated and organized corticofugal tract fibers is associated with poor long-term recovery after stroke.[6] Shelton and Reding[14] found that the probability of recovery of upper limb movement at 2 months decreased progressively with the involvement of the MC, CR, or internal capsule. In turn, Schiemanck et al[6] found that infarcts involving the internal capsule, alone or in combination with other areas, were associated with a significantly lower probability of hand motor deficit rather than infarcts in the MC, subcortex, or CR. We also found that axonal injury of the CST affected by stroke (as determined by decreased FA values in the pons) in the acute stage was only associated with PLIC damage.

Although it seems logical that larger lesions would correlate with greater deficits,[33] we found no correlation between infarct volume and motor outcome at day 90. Motor deficit was present only when critical motor regions were involved, suggesting that large lesions do not necessarily predict poor outcome and that location of the lesion might be more predictive than its size. Whereas subcortical strokes are normally smaller than cortical strokes, they are also more likely to involve both primary MC and PMC fibers, and patients with subcortical infarcts have worse motor outcome than those with cortical stroke.[14] These findings may indicate that the extent of damage specifically within the CST is a major determinant of motor deficit.

Previous structural imaging studies designed to predict motor recovery based on lesion location within the CST used conventional axial MR imaging sections and hand-drawn CST masks.[6,8] Using T2 changes to assess lesions may not accurately reflect specific neuronal damage, because lesions can be patchy and edema can contribute to T2 signal intensity hyperintensity. Conventional T2-weighted MR imaging provides excellent contrast between white and gray matter but provides no information about fiber direction.[34] In contrast, DTT clearly depicts the trajectory of the CST, making it possible to evaluate the topography and extent of tissue damage, particularly in acute stroke.[31] We found strong interrater agreement, indicating the reliability and validity of DTT as a lesion mapping technique for this purpose. Recently, some DTI studies have reported that motor outcome could be predicted by using anatomic relationships between the stroke lesion and CST damage on DTT in patients with intracerebral hemorrhage, CR infarct, and lacunar infarcts.[15-20,35] Jang et al[17] demonstrated that DTT performed at an early stage of pontine infarct (mean DTT scanning, 15 days; range, 5–30 days) is useful for predicting motor outcome. Similarly, another study reported that the degree of CST involvement on DTT within 3 days of

stroke onset was strongly correlated with the severity of motor deficit and functional recovery at 3 months in patients with an acute lenticulostriate infarct.[19] To our knowledge, ours is the first prospective study to examine consecutive patients with DTT within the first 12 hours after MCA stroke onset.

In the multiple regression analysis, the best model for predicting motor outcome at day 90 in the acute stage was PLIC damage by infarct on DWI alone (not in combination with the clinical parameters); therefore, PLIC damage could be considered an early imaging predictor of poor motor outcome. Several studies have demonstrated that the grade of initial motor deficit is the most important determinant of motor recovery.[1-5] In this respect, at day 3 we found that the clinical assessment is the most useful predictor of motor outcome and that adding information about PLIC damage increases the accuracy of the prognosis. Our findings are in line with those obtained by Feys et al[4], who analyzed the site of the lesion on CT and MR imaging between 5 and 29 days after stroke (median, 10) and obtained arm motor scores 13 to 37 days after stroke (median, 22). These authors found that arm recovery at 2 months was best predicted by a combination of the motor performance (R^2 = 59.21) and purely subcortical lesion location (R^2 = 5.31) and that motor recovery at 12 months was best predicted by clinical tests alone (R^2 = 53.11) when clinical scores were measured at 2 months after stroke.

Clinical assessment in the acute setting has some limitations. First, it can be difficult to assess the grade of paresis clinically in uncooperative or severely cognitively impaired patients, and clinical findings are occasionally inconclusive or questionable with respect to motor outcome. Second, the ischemic penumbra evidenced by perfusion-diffusion mismatch (not evaluated in the current study) can produce symptoms that are clinically indistinguishable from those produced by the infarct core.[36] The ischemic penumbra represents severely hypoperfused tissue around an infarct core; the neurons in the penumbra are supposedly structurally intact but functionally inactive, so penumbral areas are potentially salvageable.[37] In our sample, the ischemic penumbra could explain why some patients without CST involvement by infarct presented motor deficits in the acute stage and why the initial motor deficit did not correlate with motor outcome, though the effects of the ischemic penumbra are limited to the acute phase and have no direct effect on the long-term outcome. Hence, if perfusion is restored to penumbral areas and disturbances disappear (eg, at day 3) and the DWI abnormality does not involve the CST, the outcome will be good despite high m-NIHSS score on admission.

Our results show that DTT can be useful in the clinical scenario, making it possible to determine the damage to specific regions of motor pathways in patients with acute stroke consistently, easily, and quickly. Including DTT in acute stroke protocols may generate valid prognostic information because motor outcome seems strongly influenced by CST damage, in particular at the level of the PLIC. In this scenario, DTT could improve the accuracy of prognosis and help improve management in individual stroke patients.

Several limitations to our study should be emphasized. First, we considered long-term clinical follow-up (90 days) because though motor recovery seems to occur predominantly in the first few months after stroke, some patients show considerable recovery in later phases.[1] However, although several longitudinal cohort studies and randomized controlled trials found that most of the overall improvement in motor functions occurred within the first month after stroke, some degree of motor recovery continued in some patients in later phases for up to 6 months, especially in subgroups with high motor severity score on admission (59.57% of patients with motor deficit in our cohort). Second, the aim of this study was to design a simple and easy method to evaluate different CST regions qualitatively (affected or not) in the acute stroke scenario; thus, we did not consider quantitative data such as the proportion of damaged fibers, that may have improved the accuracy of our predictions.[10,11] Nevertheless, our results indicate that DTT performed within hours of stroke onset is useful for determining which patients are likely to suffer long-term motor deficits. Importantly, this approach eliminates the need for more advanced postprocessing techniques that are more time-consuming and require greater specialization, so it can be applied more widely and benefit more patients. Finally, DTI reflects the averaged water diffusion property within a voxel, which is considered an indirect indicator of the axons; therefore, this approach may oversimplify the model of the axonal structures.[31]

Conclusions

DTT should be incorporated in MR imaging protocols for acute stroke because determining the damage to specific regions of motor pathways can help predict motor outcome. Our study lends support to the idea that motor outcome is highly dependent on lesion location and the extent to which acute stroke affects the CST. In particular, PLIC damage could be considered an early imaging predictor of poor motor outcome. These findings have implications for the use of lesion mapping techniques in the prognosis of motor outcome after stroke and for establishing more effective criteria for enrolling and evaluating patients in experimental rehabilitation programs. Further research should focus on improving the accuracy of predictions of motor outcome after stroke based on early imaging predictors, with special attention to the prognostic value of DTI.

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4.4. Artículo 4. *Investigación sobre el biomarcador anisotropía fraccional*

Wallerian Degeneration in the Corticospinal Tract Evaluated by Diffusion Tensor Imaging Correlates with Motor Deficit 30 Days after Middle Cerebral Artery Ischemic Stroke. AJNR Am J Neuroradiol. 2010 Aug; 31(7):1324-30

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Wallerian Degeneration in the Corticospinal Tract Evaluated by Diffusion Tensor Imaging Correlates with Motor Deficit 30 Days after Middle Cerebral Artery Ischemic Stroke

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BACKGROUND AND PURPOSE: The quantification and clinical significance of WD in CSTs following supratentorial stroke are not well understood. We evaluated the anisotropy by using DTI and signal-intensity changes on conventional MR imaging in the CST to determine whether these findings are correlated with limb motor deficit in patients with MCA ischemic stroke.

MATERIALS AND METHODS: We studied 60 patients within 12 hours of stroke onset. At admission, day 3, and day 30 of evolution, patients underwent multimodal MR imaging, including DTI sequences. We assessed the severity of limb weakness by using the motor subindex scores (5a, 5b, 6a, 6b) of the m-NIHSS and established 3 groups: I (m-NIHSS scores of 0), II (m-NIHSS, 1–4), and III (m-NIHSS, 5–8). FA values and rFAs were measured on the affected and the unaffected CSTs in the pons.

RESULTS: FA values for the CST were significantly lower on the affected side compared with the unaffected side only at day 30 ($P < .001$), and the rFA was significantly correlated with the motor deficit at day 30 ($P < .001$; $r = -0.793$). The sensitivity, specificity, and positive and negative predictive values for motor deficit by rFA < 0.925 were 95.2%, 94.9%, 90.9%, and 97.4%, respectively.

CONCLUSIONS: WD in the CST revealed by DTI correlates with motor deficit 30 days after MCA ischemic stroke. This study highlights the utility of imaging follow-up at 30 days and the potential of DTI as a surrogate marker in clinical trials.

ABBREVIATIONS: ANOVA = one-way analysis of variance; CST = corticospinal tract; DTI = diffusion tensor imaging; DWI = diffusion-weighted imaging; EPI = echo-planar imaging; FA = fractional anisotropy; FLAIR = fluid-attenuated inversion recovery; ICC = intraclass correlation coefficient; IQ = interquartile; MCA = middle cerebral artery; NIHSS = National Institutes of Health Stroke Scale; m-NIHSS = motor subindex scores of the NIHSS; PWI = perfusion-weighted imaging; rFA = FA ratio; ROC = receiver operating characteristic; rtPA = recombinant tissue plasminogen activator; SENSE = sensitivity-encoding; WD = wallerian degeneration

Motor deficit is one of the most common sequelae of ischemic stroke, and its severity correlates with functional disability and reduced quality of life.[1] The CST is the most important motor pathway.[2] The extent of WD in the CST is one of the major determinants of motor deficit.[3] WD consists of the anterograde degeneration of axons and their myelin sheaths after proximal axonal or cell body injury from numerous causes, including stroke, and has been demonstrated through experiments, postmortem studies, transcranial magnetic stimulation, and conventional imaging.[4–8] Many studies have reported hyperintense signals regarded as WD along the affected CST on T2-weighted or diffusion-weighted imaging weeks or months after stroke, and this finding correlates well with persistent functional disability.[9–12] However, on conventional MR imaging, these findings are usually subtle and can be difficult to detect in the first few weeks; moreover, the extent of WD is difficult to quantify on conventional MR imaging and is not consistent in all patients with motor deficit.

Recently, much interest has focused on the potential of DTI for in vivo quantification of microstructural damage to cerebral white matter following stroke. DTI provides information on the predominant direction and degree of tissue water diffusion.[13,14] In the white matter, water diffuses quickly lengthwise along the fibers and slowly perpendicular to fibers, resulting in anisotropic diffusion.[15] The degree of anisotropy depends on the level of organization and integrity of the white matter tract and on the degree of freedom of water diffusion movements by oriented axonal membranes and myelin sheaths.[16] Reduced anisotropy along the CST remote from a cerebral infarct has been interpreted as WD, even when these areas appeared normal on conventional MR imaging.[17,18] Cross-sectional studies found that lower FA values in the af-

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fected CST were associated with greater motor deficit 1–2 weeks after stroke onset and a worse motor recovery at 3 months.[19–25] However, the few studies of WD of the CST determined by DTI after supratentorial stroke were performed in small heterogeneous samples months to years after stroke onset. To our knowledge, no prospective controlled studies have assessed WD in the CST immediately following MCA ischemic stroke. Therefore, we investigated the value of FA as a potential biomarker of motor deficit in relation to well-established clinical scores after MCA territory ischemic stroke.

Materials and Methods

Patients

We studied 60 consecutive patients with acute MCA hemispheric infarction within 12 hours of symptom onset. We excluded patients with cerebral hemorrhage, significant preexisting nonischemic neurologic deficit, or a history of prior stroke. The study was approved by the local ethics committee. All patients or their relatives provided written informed consent.

Clinical Assessment

Patients underwent neurologic examination, including NIHSS score, by a certified neurologist at admission, day 3, and day 30 after stroke onset. On the basis of the m-NIHSS (5a, 5b, 6a, 6b), the severity of limb weakness was categorized into grade I (total m-NIHSS score of 0), grade II (m-NIHSS, 1–4), and grade III (m-NIHSS, 5–8). All m-NIHSS assessments were administered without knowledge of the MR imaging findings.

MR Imaging

All patients underwent MR imaging examination on a 1.5T Intera scanner (Philips Healthcare, Best, the Netherlands). The protocol included axial trace DWI, FLAIR, gradient-echo T2*-weighted, PWI, time-of-flight angiography, and DTI sequences. DTI data were acquired by using single-shot EPI sequences with the SENSE parallel-imaging scheme (acceleration factor 2) after contrast agent administration. DTI with SENSE helped reduce scanning time and minimize the susceptibility and distortion artifacts typically associated with EPI sequences. Diffusion-sensitized gradients were applied along 15 noncollinear directions with a b-value of 1000 s/mm^2. Diffusion-weighted B0 images were also obtained. Other acquisition parameters were the following: TR/TE, 6795/72 ms; FOV, 23 × 23 cm; and matrix size, 112 × 112. Forty-five contiguous 3-mm axial sections covering the entire brain and brain stem were acquired parallel to the anteroposterior line. DTI scanning time was 3 minutes 10 seconds.

Data Processing

DTI data were transferred to an off-line workstation for postprocessing and visually checked for quality. Diffusion-encoded FA-weighted images were elaborated by using the calculation scheme proposed by Pajevic and Pierpaoli.[26] Color FA maps were generated following the standard convention (red, left-right; green, anteroposterior; and blue, superior-inferior). Quantitative values of FA were obtained by manually placing regions of interest on the entire CST area at the level of the rostral pons on axial sections (left and right sides) on the basis of the T2-weighted image and anatomic knowledge, by using our image display software (DTIWeb, Version 2.0, http://trueta.udg.edu/DTI/index.html).[27,28] FA values for each region of interest were obtained by averaging all voxels within the region of interest on the sides affected and unaffected by the infarct. In each patient, the FA of the CST was derived from the mean value of 3 contiguous sections. The ipsilateral-to-contralateral CST rFAs were calculated ($rFA = FA_{affected\ side} / FA_{unaffected\ side}$). FA measurements were repeated by 1 rater (J.P.) on the first 30 patients on the affected and unaffected sides (a total of 180 measurements) on 2 separate occasions 1 month apart and were performed once by 2 raters (J.P., G.B.). The presented FA values are based on the average of the mean values obtained by the 2 raters. Statistical analysis (intrarater and interrater comparisons) was performed for the mean FA values. Unaffected CSTs at the level of the rostral pons were used as internal controls for the assessment of WD-related changes because no significant differences in anisotropic diffusion are found between tracts on the left and right sides in normal subjects.[15,16] Signal-intensity abnormalities on the affected side of the CST were also determined on diffusion-weighted b=1000, T2-weighted, and FLAIR images. Finally, for infarct volumes, 2 raters (J.P., G.B.) manually outlined the areas of abnormal hyperintensity on each section of DWIs. Surfaces of areas of abnormal hyperintensity were summed and multiplied by section thickness (6 mm) and intersection gap (1 mm) to calculate infarct volumes. The results of the 2 raters were averaged.

Statistical Analysis

Data are presented as mean (± SD) values for each group. The statistical evaluation of the results was based on ANOVA with the Bonferroni correction. Levene and Bartlett tests were used to determine whether the samples had equal variances, and then ANOVA tests were applied to examine the relationship of the mean FA values on the affected side of the CST and of rFA with the clinical scores at admission, at day 3, and at day 30. To determine the association between mean FA, motor deficit at day 3 and at day 30, and signal-intensity change in affected CST, we used the ANOVA test. The Pearson rank correlation coefficient was calculated to compare the FA and rFA values with clinical scores and infarct volumes. To calculate the FA and rFA cutoff points to discriminate subjects with motor deficit at day 30 from those without, we used the ROC curves. For these tests, $P < .05$ was considered to indicate a statistically significant difference.

To compare first and second measurements of the observer number 1 (intraobserver reliability) and to compare the measurements of 2 independent observers (interobserver reliability), we used the ICC. The level of intra- and interobserver consistency was classified as fair (ICC = 0.5–0.7), good (0.7–0.9), or almost perfect (>0.90). All statistical evaluations were performed by using Minitab, Version 15.1.0.0 (Minitab, State College, Pennsylvania).

Results

Subjects

Sixty consecutive patients with nonlacunar MCA ischemic stroke (38 men, 22 women; mean age, 68 ± 13 years) were included in the study between May 2006 and December 2007. One patient missed the admission MR imaging study but completed the day 3 and day 30 studies. Two patients missed the day 3 imaging study. All patients underwent MR imaging and clinical assessment at day 30. Two patients had small slightly hyperintense areas in the periventricular white matter; none had previous strokes. The modified Rankin Scale score before the infarct was 0 in all patients.

Table 1: Summary of the demographics, diffusion tensor findings, and clinical scores

	<12 Hours (n = 59)	Day 3 (n = 58)	Day 30
Mean age		68.18 ± 13.59	
Male (%)		61	
Left-sided infarct (%)		58	
Regions involved within infarct			
Striatocapsular infarction (%)	12	6.7	6.7
Peripheral MCA territory (%)	55	45	48
Both (%)	34	48	27
Infarct volume, median (IQ range) (mL)	11.20 (7.26–98.61)	14.24 (7.25–164.04)	11.51 (9.34–120.55)
NIHSS score (mean)	11.65 ± 6.03	6.62 ± 6.13	4.37 ± 5.03
Motor score (%) (mean)			
m-NIHSS-I[a]	21.7, 0 ± 0	53.3, 0 ± 0	66.7, 0 (0)
m-NIHSS-II	31.7, 2 ± 1	25.0, 2.73 ± 1.11	15.0, 2.78 ± 1.20
m-NIHSS-III	46.7, 7.29 ± 0.99	21.7, 7.38 ± 1.04	18.3, 7.27 ± 0.99
P value	<.001	<.001	<.001
FA of CST (mean): affected, unaffected side			
m-NIHSS-I	0.578 ± 0.08, 0.597 ± 0.06	0.616 ± 0.07, 0.612 ± 0.06	0.606 ± 0.07, 0.600 ± 0.06
m-NIHSS-II	0.460 ± 0.08, 0.574 ± 0.07	0.615 ± 0.06, 0.607 ± 0.06	0.632 ± 0.06, 0.648 ± 0.08
m-NIHSS-III	0.590 ± 0.07, 0.582 ± 0.07	0.591 ± 0.07, 0.576 ± 0.07	0.421 ± 0.11, 0.600 ± 0.09
P value	.103, .014	.582, .262	<.001, .378
rFA of CST: affected, unaffected side			
m-NIHSS-I	0.967 ± 0.10	1.00 ± 0.049	1.016 ± 0.08
m-NIHSS-II	0.980 ± 0.06	1.017 ± 0.09	0.838 ± 0.13
m-NIHSS-III	1.020 ± 0.10	1.028 ± 0.06	0.697 ± 0.11
P value	.176	.641	<.001

[a] m-NIHSS indicates motor scores 5a, 5b, 6a, 6b.

Table 2: Motor scores, signal intensity on conventional MR imaging, and FA in the affected CST in patients with motor deficits at day 30

Patient, Sex	Age (Yr)	MCA Territory	Infarct Volume (mL)	NIHSS Score (0–8)	m-NIHSS Category (I-III)	FA		rFA	Hypointensity CST
						Affected	Unaffected		
1, F	83	Peripheral and striatocapsular	52.185	4	II	0.579	0.640	0.904	DWI, T2, and FLAIR
2, M	83	Peripheral and striatocapsular	75.247	8	III	0.313	0.634	0.493	None
3, F	67	Peripheral	11.320	1	II	0.559	0.611	0.914	None
4, M	47	Peripheral and striatocapsular	8.071	4	II	0.385	0.526	0.731	DWI, T2, and FLAIR
5, M	85	Peripheral and striatocapsular	11.602	3	II	0.338	0.453	0.746	DWI, T2, and FLAIR
6, M	73	Peripheral	116.460	4	II	0.480	0.698	0.687	None
7, M	76	Peripheral	34.090	3	II	0.580	0.627	0.925	None
8, F	73	Striatocapsular	12.934	7	III	0.453	0.671	0.675	DWI, T2, and FLAIR
9, M	80	Peripheral	58.758	4	II	0.427	0.648	0.658	None
10, M	68	Peripheral	120.551	8	III	0.620	0.726	0.853	DWI
11, F	67	Striatocapsular	59.456	7	III	0.396	0.663	0.597	DWI
12, M	45	Peripheral and striatocapsular	37.221	3	II	0.393	0.427	0.920	None
13, M	67	Striatocapsular	32.269	8	III	0.416	0.663	0.627	DWI
14, F	80	Peripheral and striatocapsular	116.925	8	III	0.369	0.482	0.765	None
15, M	51	Striatocapsular	50.290	5	III	0.385	0.648	0.594	None
16, M	67	Peripheral	65.331	8	III	0.310	0.485	0.639	None
17, M	67	Peripheral	78.055	2	II	0.555	0.555	1.000	None
18, F	73	Peripheral and striatocapsular	75.623	7	III	0.311	0.481	0.646	T2 and FLAIR
19, M	73	Peripheral and striatocapsular	35.135	7	III	0.400	0.536	0.746	None
20, M	68	Striatocapsular	11.967	3	II	0.504	0.621	0.811	DWI
21, M	64	Peripheral and striatocapsular	92.026	8	III	0.452	0.644	0.701	None

[a] Ranges of scores are shown in parentheses.

Clinical and Neuroimaging Characteristics

Table 1 shows the mean clinical scores and anisotropic indexes at baseline, day 3, and day 30. At day 30, mean NIHSS and m-NIHSS scores had improved compared with baseline values. At day 30, 21 patients had some motor deficit (m-NIHSS-II or m-NIHSS-III categories) (Table 2) and all patients had started physiotherapy within 2 weeks after the stroke. Infarct volumes on DWI ranged from 0.72 to 164.04 mL, and there were significant differences between the mean infarct volumes at admission, day 3, and day 30 (P = .213). The MCA territories most frequently involved were the peripheral and/or striatocapsular territories. Thirty-one patients

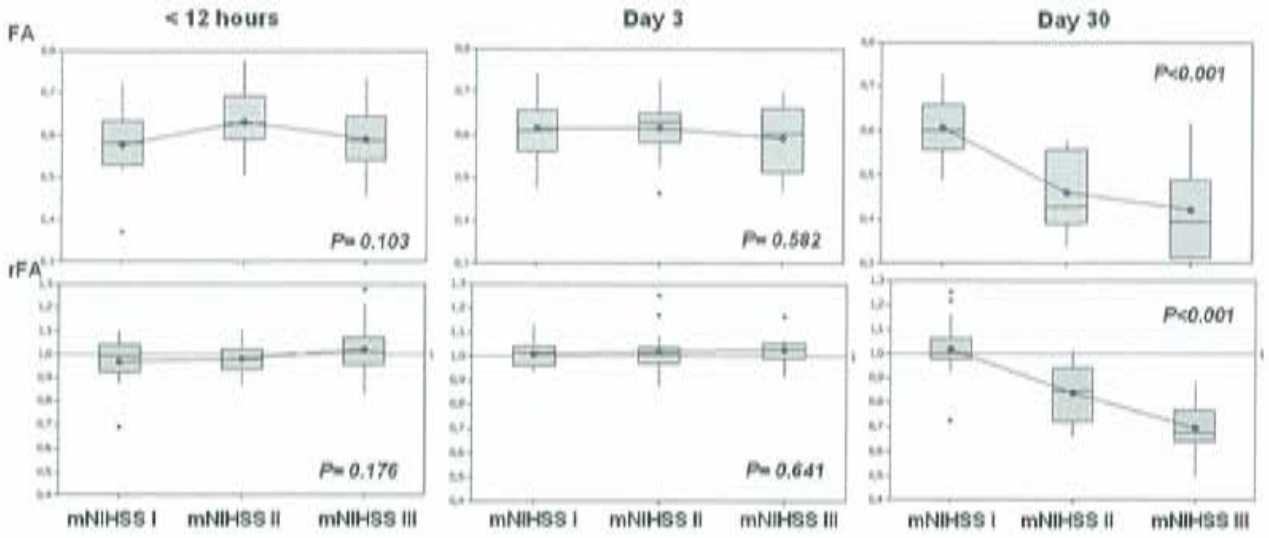

Fig 1. Evolution of mean FA values and rFAs between the affected and unaffected sides in the region of interest in the descending CST at the level of the rostral pons in the function of m-NIHSS categories at admission, day 3, and day 30. Boxplots show median values (horizontal line inside the box), quartiles (box boundaries), and the largest and smallest observed values (lines extending from the box) of FA and rFA. Anisotropy values are clearly lower in patients with motor deficits at 30 days; no differences were found between patients in the m-NIHSS-II and m-NIHSS-III groups.

(51.67%) received intravenous rtPA. There were no significant differences in rtPA treatment at admission among the m-NIHSS groups at day 30. No significant differences were found among the mean FA values in the unaffected CST in the entire group of patients at admission, day 3, and day 30 (0.609, 0.598, and 0.591, respectively) ($P = .531$). The estimated interobserver consistency showed good agreement for FA measurements (ICC = 0.809). The estimated intraobserver consistency was also good (ICC = 0.881).

Correlations Between DTI, Conventional MR Imaging Findings, and Clinical Scores

The FA and rFA cutoff points that best discriminated the presence of motor deficit at day 30 were 0.556 and 0.925, respectively. The sensitivity, specificity, and positive and negative predictive values for motor deficit by FA < 0.556 were 76.2%, 76.9%, 64%, and 85.7%, respectively. For FA at the pons ipsilateral to the infarct at day 30, the area under the ROC curve was equal to 0.897 with an error of 0.043. The sensitivity, specificity, and positive and negative predictive values for motor deficit by rFA < 0.925 were 95.2%, 94.9%, 90.9%, and 97.4%, respectively. The area under the ROC curve was equal to 0.937 with an error of 0.037.

Mean FA values on the affected side of the CST were significantly different from those on the normal contralateral side only at day 30 ($P < .001$), and mean FA values on the affected side at 30 days were lower than those at admission and at day 3 (Table 1). Likewise, rFA values in patients with motor deficits (m-NHISS-II and m-NHISS-III) were lower only at 30 days ($P < .001$). In 20 of 21 patients with motor deficits at 30 days (95.2%), FA in the affected CST was below the mean value of the group of patients without motor deficit, and FA was lower on the affected side than on the unaffected side in the group of patients with motor deficits (Table 2). FA decreased 17% in the m-NIHSS-II and 32% in NIHSS-III groups, and the decrease in mean FA and rFA values correlated negatively with the degree of motor deficit at 30 days (Figs 1 and 2). However, there were no significant differences in mean FA and rFA values between patients with NIHSS-II and NIHSS-III at 30 days.

Correlations of FA measurements in the CST at day 30 with clinical scores and infarct volumes in patients with MCA infarction are reported in Table 3. None of the clinical scores or infarct volumes measured within 12 hours correlated with FA indexes in the CST at day 30. However, clinical severity at day 3 correlated with FA indexes at day 30, and the strongest correlation was between rFA and m-NIHSS score. Moreover, no significant differences in FA values were found between affected and unaffected CST at admission or at day 3, and these FA indexes were not correlated with motor status at 30 days ($P = .292$, $r = 0.321$; $P = .231$, $r = 0.372$, respectively). On the other hand, there was no significant correlation between acute infarct volume and the NIHSS and m-NIHSS scores at day 30 ($P = .103$, $r = 0.472$; $P = .176$, $r = 0.485$, respectively).

Signal-intensity abnormalities on conventional MR imaging (hyperintensities) were detected in only 40% and 45.4% of affected CSTs in patients with m-NIHSS-II and m-NHISS-III at day 30, respectively (Fig 3). Changes in signal intensity were also associated with decreased FA and rFA values ($P < .001$). Subjects without motor deficit had no significant signal-intensity abnormalities.

Discussion

This prospective study in a relatively large cohort of patients with territorial MCA infarction found significant correlations between DTI-measured WD in the CST and m-NIHSS-measured limb motor deficit 30 days after stroke onset. Decreased anisotropy distal to the infarct on the affected side of the CST, expressed as FA values measured at the rostral pons, correlated significantly with limb motor deficit at day 30.

As far as we know, no DTI data focused on a cutoff value for WD 30 days after stroke onset have been published. Care is needed when applying cutoff anisotropic values because changes in diffusion after WD depend strongly on the preex-

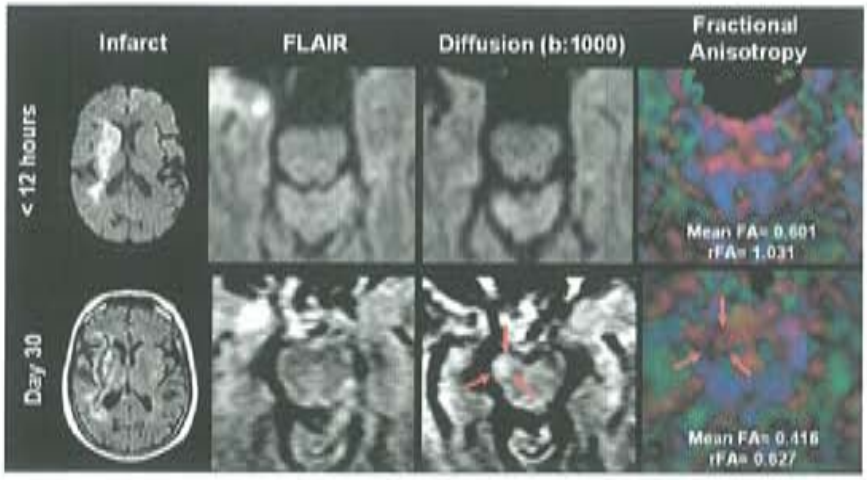

Fig 2. A 67-year-old man with acute right-sided striatocapsular infarction (upper left corner) who presented with severe hemiparesis (m-NIHSS, 8). Marked hyperintensity in the right descending CST on DWI at day 30 correlates with a decreased FA value and reduced brightness in the affected side, regarded as WD. Arrows indicate the affected right motor pathways at the level of the rostral pons.

Table 3: Correlations of affected side FA and rFA at day 30 of CST with clinical severity and infarct volume in patients with MCA stroke[a]

	<12 Hours					Day 3					Day 30		
	NIHSS	m-NIHSS	Infarct Volume	FA	rFA	NIHSS	m-NIHSS	Infarct Volume	FA	rFA	NIHSS	m-NIHSS	Infarct Volume
FA	−0.311	−0.328	−0.221[b]	0.382[b]	0.332	−0.552[b]	−0.670[b]	−0.359[b]	0.441[b]	0.081	−0.655[b]	−0.685[b]	−0.434[b]
P	<.079	<.082	.009[b]	.003[b]	.213	<.002[b]	<.001[b]	.005[b]	.003[b]	.503	<.001[b]	<.001[b]	<.001[b]
rFA	−0.308	−0.384	−0.232[b]	0.034	0.027	−0.682[b]	−0.761[b]	−0.484[b]	0.027	0.005	−0.752[b]	−0.793[b]	−0.537[b]
P	<.067	<.052	<.001[b]	.534	.838	<.001[b]	<.001[b]	<.001[b]	.871	.973	<.001[b]	<.001[b]	<.001[b]

[a] Pearson rank correlation coefficient was used for all comparisons.
[b] Parameters with $P < .05$.

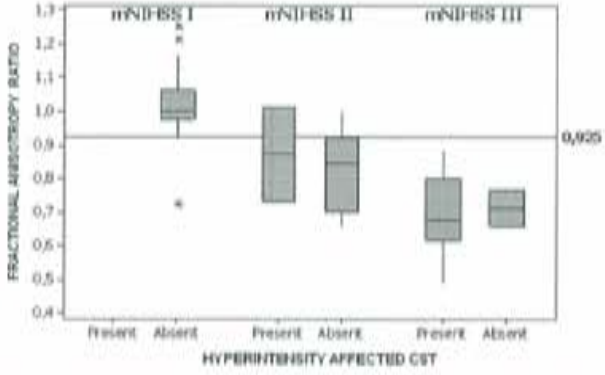

Fig 3. rFAs between the affected and unaffected CSTs are lower in patients with motor deficits at 30 days; however, fewer than half presented with abnormal signal intensities on conventional MR imaging. The line parallel to the x-axis represents the prespecified cutoff point that discriminates patients with motor deficits on the basis of rFA. The graph shows medians and quartiles.

isting architecture of the white matter.[15] Experimental studies suggest that the orientation of membranes continues to be the major determinant of anisotropy after WD. In fact, changes in diffusion observed in the rostral pons, where the CST intersects the transverse pontine fibers, differ substantially from changes observed in the cerebral peduncle or in the posterior limb of the internal capsule, where the CST consists of isolated and well-defined bundles of parallel fibers.[16] Consequently, after white matter damage, cutoff anisotropic values at different points along the CST might differ, and further studies need to clarify this point.

We propose an rFA cutoff point that discriminates a high proportion of patients with normal motor function from those with motor deficit. We found that the rFA cutoff point discriminates these patients better than the FA cutoff point. Intraindividual evaluation by using rFA can detect damage to the CST ipsilateral to the infarct in patients with high baseline FA (patient 10, Table 2). On the other hand, the trajectory of the transverse pontine fibers can introduce error in calculating CST anisotropy measured at the rostral pons, and using rFA values minimizes this error. Finally, rFA is more applicable across centers and operators than absolute FA.

In the clinical setting, DTI enables evaluation of microstructural changes in the CST better than conventional MR imaging, quantifying the amount of damage specifically within the motor system after stroke. Although WD is associated with motor deficit, motor deficit is determined by loss of function of the motor neurons and/or axons, which may or may not be accompanied by WD.

Our study shows that imaging-based regional markers correlate with motor deficits in MCA stroke. By correlating DTI findings and clinical data, we found that motor deficits 30 days after stroke increased with decreased anisotropy in the pons. The decrease in the FA values in the affected CST becomes progressively smaller during the subacute-to-chronic stages of stroke, and previous studies have demonstrated worse outcome in patients with evident WD on conventional MR imaging techniques.[8-12] Signal-intensity abnormalities related to WD are generally not detected until 4 weeks after stroke, when the main finding is a hyperintensity along the affected tracts on

DWI and T2-weighted images. After assessing WD changes qualitatively on conventional images and quantitatively by using DTI, we found differences in anisotropy in patients with motor deficits who had no signal-intensity abnormalities on conventional MR imaging. Most important, most patients with motor deficits at 30 days who presented decreased FA values had no signal-intensity abnormalities on the affected side of the CST; more specifically, fewer than half presented hyperintensities on FLAIR and/or restricted diffusion on DWI. The signal-intensity change in the affected CST was strongly associated with lower FA indexes. These findings suggest that DTI is more sensitive in detecting tissue changes regarded as WD than conventional MR imaging and DWI.

In an experimental model after unilateral MCA occlusion in rats, signs of WD in the CST were detected in the brain stem in histologic stains as early as 2–7 days after the stroke.[4] Within the second stage, WD decreased gradually up to 6 weeks. Other recent studies have demonstrated early signs of WD in patients with stroke within 2–3 weeks of onset by using DTI.[16,24] Greater FA reduction along the CST on the affected side after cerebral infarction is associated with greater early motor deficit and worse motor recovery at 3 months.[19-22] In our study, we found significant correlations between FA indexes and motor deficit only at 30 days, and the reductions in mean FA, 17% and 32% in patients with m-NIHSS-II and m-NIHSS-III respectively, are similar to other published values measured at the pons.[15,18] Thomalla et al.[17] found a 13% decrease in FA measured at the cerebral peduncle 2–6 months and 2–16 days after stroke.

Severe WD in the CST distal to a supratentorial infarct in the acute stage has been regarded as a predictor of worse motor outcome.[17,23] Liang et al[24] conducted a similar longitudinal controlled study by using serial DTI to assess both antegrade and retrograde WD in 12 patients with subcortical ischemic infarctions involving the posterior limb of the internal capsule. They found progressive decrease in FA with time starting from the first week in the region just proximal and distal to the internal capsule lesion. On the other hand, Wanatabe et al[25] used serial DTI evaluations to assess WD in the CST of 16 patients with stroke (6 ischemic, 10 hemorrhagic). They found that the good recovery group had no significant change in anisotropy in the rostral pons between 2 and 3 weeks, whereas the poor recovery group had a significant decrease in anisotropy during that time. Although not specifically calculated, Fig 4 of their study shows that the 0.9 ratio is the approximate anisotropy cutoff for distinguishing the separate recovery groups 3 weeks after onset.

Recently, Kusano et al[29] reported that FA indexes measured in the cerebral peduncles within 2 days after intracerebral hemorrhage onset may predict functional motor outcome. In this study, a region of interest–based analysis of the FA of the CST in the cerebral peduncles showed that FA measured in the affected side decreased by 11% compared with the unaffected cerebral peduncle. The cutoff point of the rFA for the good (m-NIHSS, 0–2) and poor (m-NIHSS scores ≥ 3) outcomes was 0.85. Despite the differences in the study design, disease, and time of evolution when the cutoff point was determined, our cutoff point of 0.925 at 30 days is very similar to theirs.

We found no earlier changes in DTI that predicted motor outcome. The 3-day follow-up included in our protocol failed to show changes in anisotropy at a point in the CST far from the infarct, specifically at the rostral pons. Placing the region of interest nearer to the infarct might find changes in FA values at day 3 that could predict poor motor outcome.

A valid surrogate functional outcome measure for stroke is needed. Clinical variables such as age and initial stroke severity measured with a neurologic deficit scale such as the NIHSS have consistently been associated with functional outcome after ischemic stroke.[30] However, previous studies have demonstrated that infarct volume on conventional MR imaging correlates only modestly with functional outcome.[31] We found that stroke severity at day 3 correlated negatively with rFA at day 30; however, this correlation was not found during the first 12 hours after onset. In the acute phase (<12 hours after onset), three-quarters of our patients had motor deficits, and they were moderate or severe (m-NIHSS-III) in nearly half of these patients. However, at day 3, only half of our patients had motor deficits and only 20% of these deficits were moderate or severe. These differences in motor deficits between the acute phase and day 3 are probably due to the ischemic penumbra (not evaluated in our study).

On the other hand, stroke volume measured on conventional MR imaging at day 3 and at day 30 correlated weakly with FA indexes at day 30. Volume measures do not take into account the location of the lesion or the functional pathways involved, and 2 different infarcts of the same size in different locations could have very different functional expression. Thus, rather than total stroke lesion volume, it seems much more reliable to use DTI to evaluate the extent of damage specifically within the CST to determine motor deficit and axonal injury. However, we found no evidence that stroke severity at day 3 was expressed as WD. Placing the region of interest in a region of the CST closer to the infarct might make it possible to detect changes in FA indexes earlier and to predict which patients will have worse outcomes in the chronic phase so that rehabilitation interventions can be adjusted to each patient's need from the earliest stages to use health care resources more efficiently.

One new research line aims to improve neurologic outcome through stem cell–based treatment of chronic stroke.[32] Although the study of the mechanisms underlying stem cell–based treatment has focused on angiogenesis and neurogenesis, white matter reorganization may contribute to functional recovery after stroke. The earliest stem cell transplantations were carried out 4–9 weeks from onset,[33] and 4 weeks was also the time when our study demonstrated CST damage in the patients with motor deficits. DTI may also be useful in determining the real state of the CST before treatment, quantifying the amount of damage specifically within the motor system after stroke, and FA indexes could be an alternative way of monitoring the changes in cerebral tissue that lead to improved outcome.

Several potential limitations to our study merit comment. First, manual region-of-interest placement is subject to operator bias, especially at day 30, when CST damage is present, and this may result in variability in location, size, and shape. Ideally, all the descending motor pathway areas should have been included to avoid underestimating the degree of DTI change and thus the degree of WD. Further studies by using automated region-of-interest analysis or voxel-based analysis

may resolve this issue. Using m-NIHSS categorization does not diminish the significance of the association between decreased FA and motor deficit, though dedicated neuromotor test batteries like the Medical Research Council Scale or Motricity Index might find significant anisotropic differences between different grades of motor deficits. On the other hand, it could be interesting to determine other anisotropic parameters, like mean diffusivity or diffusion tensor eigenvalues, and to analyze whether changes in the affected tract can be detected earlier to enable better and earlier prognosis. Finally, the small number of patients with motor deficits at 30 days could be a limitation; however, our sample size seems sufficient given that the profile of patients defined by the inclusion criteria is similar, the variability in the findings for the unaffected side among all patients is low, and the normal unaffected side served as an internal control that minimized the potential variability among patients. Nevertheless, a larger sample would have increased the power of our findings.

Conclusions

In summary, the current study shows that DTI is more sensitive than conventional MR imaging in detecting WD and provides further evidence that FA could potentially be used as an imaging surrogate marker for motor deficit. To our knowledge, this is the first study to determine an anisotropic cutoff point in the evaluation of WD in patients with motor deficits 30 days after stroke. Identifying imaging biomarkers of WD represents a real challenge and could have implications for clinical decision-making. Evaluation of CST integrity by using anisotropic parameters can be useful for setting therapeutic goals and for selecting and monitoring patients with stroke for individual rehabilitation strategies.

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5. Síntesis de los resultados y discusión.

5.1 Aportaciones específicas de las publicaciones del estudio.

Investigación base sobre biomarcadores de imagen por RM en infarto cerebral.

Este trabajo se aportaba la definición de un panel de 7 criterios para validar un biomarcador radiológico de RM en el infarto cerebral (BRIC-RM). Estos criterios se detallan en la tabla 1.

TABLA 1. Panel de criterios para validar un BRIC-RM

1. Debe ser un cambio biológico, fisiológico, bioquímico o anatómico detectable por RM.
2. El biomarcador radiológico por RM debe estar muy relacionado con la diana del tratamiento.
3. El biomarcador radiológico por RM debe tener una relación lógica con la severidad de la enfermedad. Es importante tener una relación fuerte con el marcador final de evolución clínica.
4. Debe ser posible medir y cuantificar el biomarcador de una manera precisa, reproducible y fiable.
5. Los nuevos tratamientos alteran el valor del biomarcador radiológico por RM. Esta variación se relaciona con el éxito o el fracaso del tratamiento evaluado y con la evolución clínica final
6. El biomarcador radiológico por RM puede dar información de la toxicidad del tratamiento.
7. Existen publicaciones con evidencia científica alta que apoyan su uso clínico en infarto cerebral. La existencia de estudios preclínicos sería muy útil.

BRIC-RM: Biomarcador radiológico de infarto cerebral por RM.

. En este trabajo se aporta también una selección de 8 biomarcadores radiológicos en el infarto cerebral por RM (BRIC-RM) que pueden ser más útiles en el infarto cerebral. La definición de los 8 BRIC se detalla en la tabla 2.

TABLA 2. Propuesta de BRIC-RM

A. PERMEABILIAD ARTERIAL
- Permeabilidad vascular inicial y final. Recanalización inicial o tardía.
B. VOLUMEN DEL INFARTO
- Volumen inicial y final del infarto. Variación de volumen en los primeros 3 días o en el primer mes.
C. REVERSIBILIDAD DEL INFARTO CEREBRAL
- Reversibilidad de la lesión en difusión.
D. ALTERACIÓN DE LA PERFUSION
- Trastorno inicial y final de la perfusión. Reperfusión precoz y tardía.
E, F, G. VOLUMEN DE PENUMBRA O TEJIDO EN RIESGO.
- Diferencia entre Difusión y Perfusión.
- Diferencia entre Difusión y estado clínico.
- Diferencia entre Difusión y permeabilidad vascular.
H. TRANSFORMACION HEMORRÁGICA.
Grados de transformación hemorrágica.

BRIC-RM: Biomarcador radiológico de infarto cerebral por RM.

. Finalmente se realiza una revisión de la validez de los 8 Biomarcadores de imagen por RM en el infarto cerebral (BRIC). La valoración de cada uno se resume en la tabla 4

TABLA 3. Validez de cada biomarcador radiológico del infarto cerebral (BRIC).

	Oclusión Vascular	Volumen Difusión	Reversibilidad de Difusión	Alteración de Perfusión	Diferencia Difusión Perfusión	Diferencia Difusión Clínica	Diferencia Difusión Angio	Transformación hemorrágica
Visible por RM	+++	+++	+++	+++	+++	+++	+++	+++
Diana	+++			++	++	++	++	
Correlación con severidad	+	+	+	+	+	+	+	+
Cuantificable	+	+	+	+	+	+	+	+
Cambia con el tratamiento	+++	++	++	+++	+++	+++	+++	++
Valora toxicidad								+++
Evidencia en publicaciones	+	+	+	+	+	+	+	+

RM: Resonancia Magnética. BRIC: Biomarcador Radiológico del infarto cerebral.

+++ Correlación Alta, ++ Correlación Moderada, + Correlación Leve

Esta revisión de los BRIC-RM fue pionera en el momento de su publicación y fue la base de la realización de estudios posteriores de nuestro grupo en la validación de BRIC-RM específicos.

En estudios posteriores de revisión sobre el tema (29) se ha diferenciado las utilidades de los BRIC-RN en cuatro áreas: biomarcador cuantificable de manera fiable, biomarcador que pronostica la evolución clínica final, biomarcador que predice la probabilidad de respuesta al tratamiento y biomarcador que puede ser marcador surrogado sustituyendo a los "endpoint" clínicos clásicos en los ensayos clínicos.

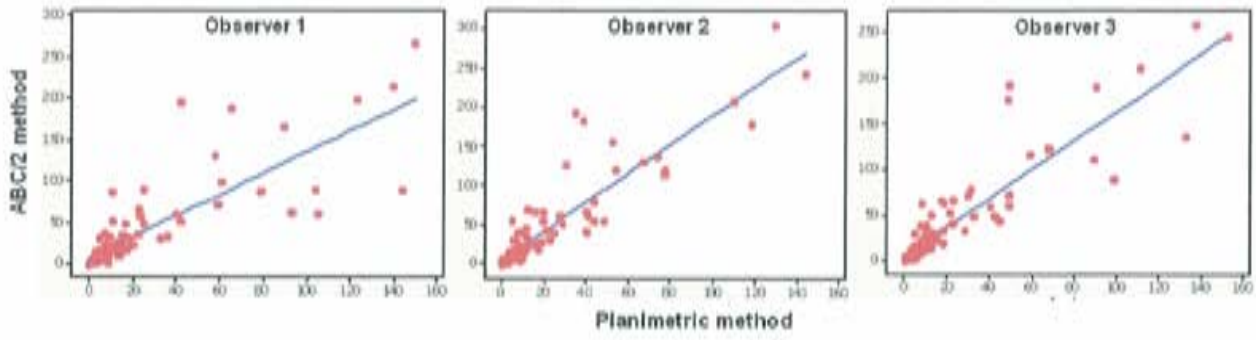
En esta revisión (29) se ha conservado la misma relación de biomarcadores pero agrupándolos en cinco categorías: BRIC-RM de la lesión isquémica, del compromiso hemodinámico, de la permeabilidad vascular y de la transformación hemorrágica.

De todas maneras ese artículo confirma la vigencia del análisis de los puntos débiles y fuertes de cada uno de los biomarcadores que aparece en la tabla 3 de la tesis.

Investigación sobre el biomarcador volumen del infarto cerebral

En esta investigación se pretende analizar la fiabilidad del método de cuantificación del volumen del infarto cerebral en la secuencia de difusión en la RM. En este trabajo se confirmó la existencia de una buena correlación entre los valores de volumen del infarto determinados por cada técnica en cada paciente como se muestra en la figura 4. Es decir que cuando el volumen era alto o bajo, era alto o bajo para los dos métodos de cuantificación

FIGURA 4. Correlación entre el valor del volumen por los dos métodos en cada observador.



Por otro lado, se demostró que la reproducibilidad era muy alta con una variabilidad interobservador bajo. De modo concreto el coeficiente de correlación intraclase era de 0.992 para el método ABC/2 y de 0.985 para el método planimétrico.

Sin embargo este trabajo también demostró la existencia de una diferencia clara en el valor concreto del volumen del infarto por los dos métodos para cada observador con una tendencia a sobreestimación por el método ABC/2 (TABLA 4)

TABLA 4. Volumen del infarto por cada observador con método ABC/2 y volumétrico.

Método	Observador	Media+/- SD	Q1	M	Q3
ABC/2	1	39.20±53.67	8.40	17.57	49.23
	2	45.60±60.09	8.07	21.87	55.71
	3	42.53±56.57	8.13	18.50	54.02
Planimétrico	1	24.30±34.81	5.21	10.36	22.83
	2	21.74±29.51	4.22	9.84	25.46
	3	23.46±32.56	4.95	10.02	24.52

Todo los valores se expresan en cc Media+/- DE: Volumen medio con desviación standard, Q1: 1r^{l} Cuartil, M: Mediana, Q3: 3^{r} Cuartil.

En esta investigación se demostró las diferencias del volumen entre ambos métodos en función del territorio vascular. En la tabla 5 se confirma que los volúmenes obtenidos con el método ABC/2 (19.34 cc [8.20, 52.94]) (mediana, 1rQ, 3rQ) eran significativamente mayores que los volúmenes obtenidos con el método planimétrico (9.93 cc [4.92, 23.09]) ($p < 0.0001$). En resumen, la técnica ABC/2 sobreestima el volumen del infarto cerebral con una media de incremento falso (volumen con ABC/2 – volumen planimétrico) de 7.33 cm3 [1.29, 22.17], lo cual representa un aumento (volumen con ABC/2 / volumen planimétrico) del 162.56 % [121.70, 248.52] sobre el valor del volumen real determinado por método planimétrico.

TABLA 5. Diferencias de volumen del infarto según el territorio de ACM para el método ABC/2 y planimétrico.

Territorio de ACM	ABC/2	PLANIMETRICO	Correlación	slope	R^2
Superficial	9.04 [2.00;15.98]	5.350 [1.88;9.21]	0.83	1.62	69.4%
Profundo	21.38 [8.92;50.64]	9.84 [5.13;22.49]	0.86	1.62	75.4%
Superficial + Profundo	61.2 [23.4;130.8]	40.09 [14.93;72.81]	0.79	1.29	63.0%
Global	19.34 [8.20;52.94]	9.93 [4.92;23.09]	0.86	1.52	75.4%

Todos los valores en cc. Media.

En la discusión de la publicación se comenta que el impacto clínico real de esta sobreestimación es un tema conflictivo con discrepancia entre diversos autores (19, 30, 31) y con propuestas de que quizás no tenga un impacto clínico real. De todos modos estos resultados apoyan la necesidad de desarrollar métodos fiables de cuantificación automática y rápida del volumen del infarto cerebral que puedan aplicarse en fase aguda del infarto cerebral.

Posteriormente a la publicación de esta investigación han aparecido diversos trabajos con resultados contradictorios. Por un lado (32) se estudia la aplicación del método ABC/2 en la cuantificación de la penumbra por CT perfusión. Los autores demuestran que es una técnica reproducible entre diversos autores lo cual coincide con los resultados de nuestra publicación pero no comparan los resultados con el método planimétrico considerada el patrón oro. Estudios futuros deberán abordar este tema.

En otro trabajo (33) los autores estudian la fiabilidad del método ABC/2 para cuantificar la penumbra por RM mediante la comparación con el método planimétrico. Este trabajo se apoya en nuestra publicación para corregir dos de las limitaciones; por un lado la necesidad de estudiar infartos de mayor volumen y por lado la necesidad de estudiar la penumbra. Los autores confirman la correlación y reproducibilidad de la técnica que apuntábamos en nuestra publicación pero encuentran que ABC/2 sí que es una técnica fiable para medir la penumbra cuando se usa como criterio de penumbra la existencia de una diferencia de 50 cc entre Difusión y Perfusión. Es decir progresivamente se está definiendo mejor la utilidad de la técnica ABC/2 y estudios futuros deberán seguir

analizando la correlación con el método planimétrico en cada área de estudio de los pacientes con infarto cerebral.

Investigación sobre el biomarcador tractografía

En esta investigación se pretendía analizar la capacidad pronóstica de la topografía de afectación por el infarto del tractograma de TCE. Esta investigación fue el primer estudio prospectivo controlado que examinaba pacientes con TTD en las primeras 12 horas de evolución. Los estudios previos se basaban en secuencias convencionales T2 que no permiten valorar el TCE (32, 35, 36).

En este trabajo se demostró que la existencia de una afectación del TCE en el brazo posterior de la cápsula interna (BPCI) era el biomarcador con mayor poder predictivo de la evolución motora final y también del daño axonal y de la severidad clínica en el ingreso ($P < 0.001$).

También se determinó la ausencia de correlación entre el volumen del infarto y el estatus motor en el día 90 ($P=0.176$, $r=0.485$). Es decir, la presencia de un infarto de gran volumen no implica per se un mal pronóstico de la función motora del paciente. Por ejemplo los infartos subcorticales son más pequeños que los corticales pero con más probabilidad pueden afectar a las fibras motoras primarias y secundarias y dar un déficit motor importante. Estos datos indican que el grado de extensión del infarto sobre el TCE es el mayor determinante de déficit motor del paciente.

El trabajo demostró (tabla 6) que la afectación del TCE a nivel del BPCI en el estudio inicial es el biomarcador asociado a la mayor sensibilidad, especificidad, valor

TABLA 6. Sensibilidad, Especificidad, Valor Predictivo Positivo y Valor Predictivo negativo del grado de déficit motor según el nivel de afectación de la cápsula interna.

	Evolución Función Motora	m-NIHSS	Sensibilidad	Especificidad	VPP	VPN
BPCI < 12 horas	Día 30	I vs II/III	66.67	100.00	100.00	84.78
		II vs III	100.00	70.00	78.57	100.00
	Día 90	**I vs II/III**	**73.68**	**100.00**	**100.00**	**89.13**
		II vs III	**100.00**	**71.43**	**85.71**	**100.00**
BPCI el dia3						
	Día 30	I vs II/III	71.43	100.00	100.00	86.67
		II vs III	100.00	60.00	73.33	100.00
	Día 90	I vs II/III	78.95	100.00	100.00	91.11
		II vs III	100.00	57.14	80.00	100.00
SC el dia 3	Día 30	I vs II/III	68.75	87.18	68.75	77.27
		II vs III	54.55	50.00	54.55	50.00
	Día 90	I vs II/III	47.37	82.93	56.25	77.27
		II vs III	50.00	57.14	66.67	40.00
CR el dia 3	Día 30	I vs II/III	71.43	56.41	46.88	78.57
		II vs III	63.64	20.00	46.67	33.33
	Día 90	I vs II/III	73.68	56.10	43.75	82.14
		II vs III	58.33	0.00	50.00	0.00

BPCI: Brazo posterior de cápsula interna, SC: Área subcortical, CR: Corona radiata, VPP: Valor Predictivo Positivo, Valor Predictivo Negativo.

predictivo positivo y valor predictivo negativo para predecir el nivel de déficit motor en el día 90 (73.7%, 100%, 100%, and 89.1%, respectivamente). Además se determinó que tenía mayor poder predictivo que la misma valoración clínica inicial para predecir la función motora a los 3 meses (R2=75.50, F=80.09, P<0.001).

Este trabajo comparó los diferentes modelos predictivos (Tabla 7) y demostró que en las primeras horas la afectación del BPCI aporta el 75.5% de la varianza en la predicción de la evolución. Por el contrario en el día 3 la valoración clínica aporta el 79% de la capacidad predictiva y el biomarcador radiológico solo el 6.62%. En el día 30 la valoración clínica aporta el 90.10 del poder predictivo. Esta investigación es la primera que demuestra que la determinación de un biomarcador radiológico como la afectación del TCE en el BPCI es el mejor modelo predictivo en las primeras 12 horas de evolución de la evolución motora final. Los estudios previos solo demostraban la utilidad de la valoración clínica (20, 22, 37, 38, 39). En este sentido nuestra investigación confirma que en el día 3 del infarto la valoración de la escala clínica es el mejor predictor y que la asociación al biomarcador "afectación de BPCI" aumenta el poder pronóstico. De todas maneras la valoración clínica tiene limitaciones (40) y es importante tener un método objetivo radiológico. Es decir los datos de esta investigación sugieren que sería útil añadir la TTD en el protocolo radiológico de un paciente con infarto para mejorar su manejo clínico.

TABLA 7. Modelos seleccionados para el modelo de regresión múltiple para predecir el valor de mNIHSS al día 90 del infarto

Modelos predictores de función motora final	B	t-value	Added R^2
Variables medidas en las primeras 12 horas (R^2=75.50, F=80.09***)			
Afectación de BPCI	5.36	8.95***	75.50
Constante	0.64		
Variables medidas en día 3 (R^2=85.62, F=74.39***)			
m-NIHSS	0.75	5.72***	79.00
Afectación de BPCI	2.28	3.39**	6.62
Constante	-1.58		
Variables medidas en día 30 (R^2=90.10, F=236.72***)			
m-NIHSS	0.96	15.39***	90.10
Constante	-0.40		

B=Coeficiente de regresión *** P<0.001; ** P<0.01; * P<0.05.

Posteriormente a la publicación de esta investigación han aparecido diversos trabajos con resultados relacionados. Unos autores se basan en esta publicación como base y desarrollan un método de cuantificación del TCE basado en el análisis de voxel (41) que demuestra su utilidad para predecir el déficit motor en extremidades superiores aunque todavía supone una técnica mucho más compleja e imposible de aplicar en la

fase aguda a diferencia de la metodología que se propone en esta tesis. Estudios futuros deberán validar esta nueva técnica de cuantificación de la tractografía.

Otro autor (42) demuestran la existencia de una reducción de la anisotropía del BPCI se relaciona con una mayor actividad cortical compensadora en el estudio funcional cerebral y con una peor evolución de la función motora. Por todo ello proponen la AF del BPCI como un nuevo biomarcador predictor de la recuperación de la función motora con tratamiento rehabilitador.

Otros autores usan de manera combinada la RM funcional con la TTD (43) para demostrar que la disminución de FA del BPCI se asocia a un aumento de la actividad cortical contralateral. Futuros estudios combinando estas dos biomarcadores pueden predecir mejor la respuesta a tratamiento rehabilitador.

Investigación sobre el biomarcador anisotropía fraccional

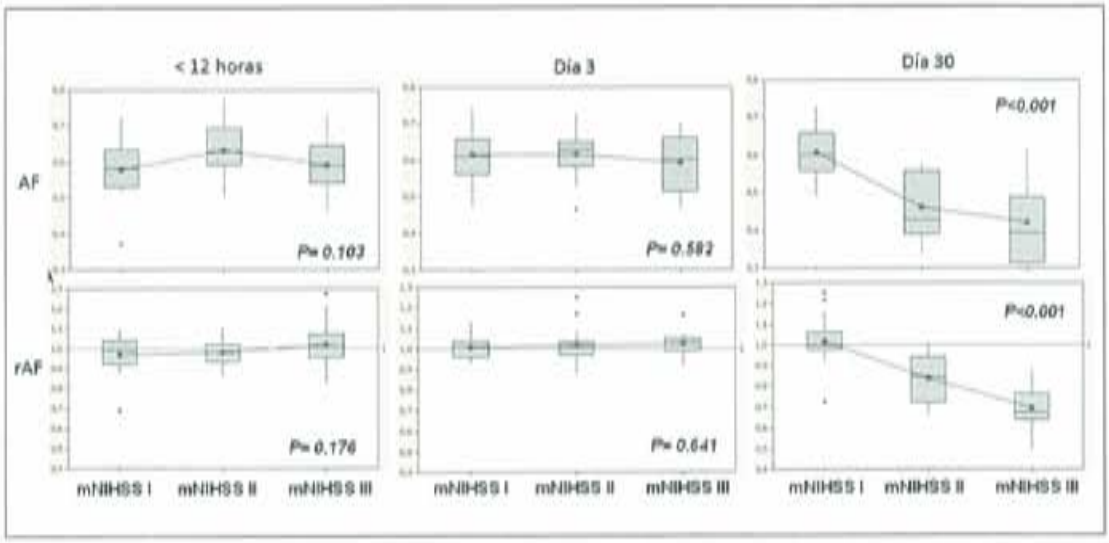
En esta investigación se pretendía analizar la capacidad pronóstica del biomarcador valor de AF de TCE. La primera aportación del trabajo fue demostrar la reproducibilidad del biomarcador con escasa variabilidad intraobservador (K: 0.78) e interobservador (K: 0.76).

El trabajo demostró que el valor de AF en el día 30 estaba reducido de manera significativa y sobretodo en pacientes con déficit motor significativo determinado por unos grados mNIHSS II y mNIHSS III de déficit motor (TABLA 8 y Figura 5).

TABLA 8. Datos Radiológicos y Clínicos

	< 12 horas (n=59)	Día 3 (n=58)	Día 30 (n=60)
Score Motor, %, media ± DS			
m-NIHSS I	21.7, 0 ± 0	53.3, 0 ± 0	66.7, 0 (0)
m-NIHSS II	31.7, 2 ± 1	25.0, 2.73 ± 1.11	15.0, 2.78 ± 1.20
m-NIHSS III	46.7, 7.29 ± 0.98	21.7, 7.38 ± 1.04	18.3, 7.27 ± 0.90
p-value	*P*< .001	*P*< .001	*P* <0.001
AF de TCE, media ± DE: afectado, lado no afectado			
m-NIHSS I	0.578 ±0.08, 0.597±0.06	0.616 ± 0.07, 0.612 ±0.06	0.606 ±0.07, 0.600 ±0.06
m-NIHSS II	0.460 ±0.08, 0.574 ±0.07	0.615 ±0.06, 0.607 ±0.06	0.632 ±0.06, 0.648 ±0.08
m-NIHSS III	0.590 ±0.07, 0.582 ±0.07	0.591 ±0.07, 0.576 ±0.07	0.421 ±0.11, 0.600 ±0.09
p-value	*P* = .103 *P* = .014	*P* = .582 *P* = .262	*P* < .001 *P* = .378
Ratio de AF del TCE ± DE: afectado, lado no afectado			
m-NIHSS I	0.967 ±0.10	1.00 ±0.049	1.016 ±0.08
m-NIHSS II	0.980 ±0.06	1.017 ±0.09	0.838 ±0.13
m-NIHSS III	1.020 ±0.10	1.028 ±0.06	0.697 ±0.11
p-value	*P* = .176	*P* = .641	*P* < .001

FIGURA 5. Evolución de la AF y rAF en estudio inicial día 3 i día 30.



El trabajo confirmaba que los signos clásicos de degeneración walleriana (DW) con hiperintensidad en FLAIR se asociaba a la existencia de una disminución de los valores de AF y rAF en ese TCE.

La investigación realizó una aportación significativa al determinar que los valores de corte ("cut-off") de la AF y de la rAF en el día 30 para identificar a los pacientes con un déficit motor severo en el día 30 eran de 0.526 y de 0.89. Estos valores coinciden parcialmente con los aportados por otra publicaciones (44) que habían sugerido un valor de corte de 0.85. Estudios futuros multicéntricos de rehabilitación pueden usar este valor de corte del biomarcador rAF de TCE.

Este trabajo también demostró que el biomarcador volumen del infarto el día 30 se correlaciona muy débilmente con el grado de déficit motor en el mismo día 30 a diferencia de rAF que muestra una correlación mucho mejor. Estos datos coinciden con los resultados de otros trabajos (25, 26, 28,45) y supone la validación del rAF como biomarcador con utilidad como marcador subrogado en futuros estudios en lugar de la valoración clínica. Esta confirmación es importante porque supone validar un biomarcador que podría ser utilizado como marcador surrogado funcional ofreciendo una alternativa válida a los "endpoints" clínicos establecidos. Estudios futuros podrán validar esta estrategia.

Posteriormente a la publicación de esta investigación apareció una publicación que proponía que la reducción del FA se puede ya demostrar mucho más precozmente en el tercer día de evolución (46, 47) lo cual no coincide con nuestro hallazgo de que no se apreciaban este signo de DW hasta el día 30 de evolución. Estudios futuros con

estudios entre el día 3 y el 30 pueden confirmar la aparición más precoz de signos de DW.

Finalmente en nuestro grupo de trabajo se ha realizado otro proyecto de investigación para determinar la capacidad predictiva a largo plazo de la AF del TCE (48)

5.2 Limitaciones específicas de las investigaciones realizadas.

Cada una de las investigaciones de esta tesis tiene limitaciones

Investigación de base sobre biomarcadores de imagen

El primer artículo de la tesis es un trabajo pionero en el estudio de los biomarcadores radiológicos pero tiene varias posibles limitaciones. La primera es que no analiza todos los posibles biomarcadores por RM. En este sentido en esta tesis se realizaron otras dos líneas de investigación para analizar la validez de la tractografía y de la anisotropía fraccional en el infarto. Tampoco se han analizado biomarcadores de otras pruebas radiológicas o la relación con los biomarcadores moleculares dado que estaban fuera del objetivo de la publicación.

Investigación sobre el biomarcador volumen del infarto cerebral

La línea de investigación sobre el volumen tiene varias limitaciones. La primera es que se estudió un grupo de infartos de pequeño volumen (19.34 cc [8.20, 52.94]). Estudios futuros tendrán que confirmar los hallazgos en infartos de mayor tamaño.

Una segunda limitación es que no se ha analizado la validez del método ABC/2 para estudio de la penumbra isquémica. Estudios futuros tendrán que analizar la validez de este método para medir este biomarcador.

Una tercera limitación es que no se ha analizado el valor terapéutico del método ABC/2. Estudios futuros tendrán que analizar la validez de nuevos métodos volumétricos con respecto al método ABC/2.

Una cuarta limitación es que solo se han estudiado infarto de ACM que son los que usualmente se incluyen en los ensayos clínicos. De todas maneras, los estudios futuros tendrán que analizar la validez de nuevos métodos volumétricos en infartos de otros territorios.

Investigación sobre el biomarcador tractografía

La línea de investigación sobre la tractografía tiene varias limitaciones. La primera es que se ha valorado el día 90 para determinar el grado de función motora final aunque algunos pacientes pueden mostrar una mejoría más allá del 6º mes. Por ello se ha realizado otro estudio del grupo para valorar la evolución final a los dos años.

La segunda limitación es que solo se ha realizado una valoración cualitativa de la afectación del TCE y es posible que la cuantificación de otros biomarcadores como la densidad de fibras pudiera ser útil. Estudios futuros pueden determinar la utilidad de estos biomarcadores cuantitativos del estado de TCE. Sin embargo es importante subrayar que la valoración cualitativa tiene la gran ventaja de que se puede hacer por cualquier especialista en fase aguda sin necesidad de postproceso complejo.

La tercera limitación es que la reconstrucción del TCE a partir de un pixel da una visión indirecta de los axones lo cual puede simplificar la estructura real de los axones. Estudios futuros pueden confirmar los resultados de este estudio con otras técnicas de reconstrucción tractográfica.

Investigación sobre el biomarcador anisotropía fraccional

La línea de investigación sobre anisotropía fraccional tiene varias limitaciones. La primera es que la realización de medidas mediante la realización de un ROI puede inducir errores. Los estudios futuros con colación automática de ROI y análisis por voxel puede evitar esta limitación.

La segunda limitación es que se ha usado una escala motora simplificada basada en el la escala NIHSS. El uso de estudios futuros de escalas especializadas como el índice de motricidad puede encontrar diferencias en la anisotropía de diferentes grados de déficit motor.

La tercera limitación es que se ha usado solo la AF como biomarcador de la ITD pero estudios futuros puedes estudiar si otros biomarcadores como la difusitividad media o los valores eigen puedan detectar más precozmente la degeneración walleriana (DW).

La última limitación es el pequeño número de pacientes con déficit motor en el da 30. Sin embargo todos los pacientes tenían la homogeneidad proporcionada por cumplir los mismos criterios de inclusión y además se ha comparado en cada paciente la mitad del bulbo con DW con respecto a la mitad normal para minimizar la variabilidad. De todas

maneras estudios futuros con un tamaño muestral mayor puede mejorar la validación de la AF como biomarcador

5.3 Aportaciones globales de las publicaciones del estudio.

Esta tesis aporta los siguientes resultados globales:

- Creación de un panel de criterios de validación de los Biomarcadores de imagen por RM en el infarto cerebral.
- Propuesta de una batería de 8 grupos principales de Biomarcadores en el infarto en fase aguda por RM en fase aguda.
- Análisis de los puntos fuertes y débiles de cada uno de los principales BRIC-RM.
- Demostración de la alta correlación entre los métodos ABC/2 y planimétrico para cálculo del volumen del infarto cerebral por RM.
- Demostración de que la buena reproducibilidad del método ABC/2 para cálculo del volumen del infarto cerebral por RM.
- Demostración de que el método ABC/2 sobrestima el volumen real del infarto. Este dato limita su utilidad clínica y su aplicación en ensayos clínicos futuros.
- Demostración de que la afectación del TCE a nivel del BPCI en las primeras 12 horas de evolución muestra una fuerte asociación con el déficit motor severo en las primeras 12 horas y con la una función motora pobre el día 90.

- Demostración de que la ausencia de correlación entre el volumen del infarto y el estatus motor en el día 90. Es decir, la afectación del TCE es el mayor determinante de déficit motor.

- Demostración de la validez de una valoración cualitativa del TCE que puede hacerse por cualquier especialista en fase aguda sin necesidad de postproceso complejo.

- Demostración de que la TDT debe incorporarse a los protocolos radiológicos de RM del infarto cerebral agudo para predecir en fase aguda la evolución motora final.

- Demostración de que la ITF es más sensible que la RM convencional en la detección de la degeneración walleriana.

- Validación de AF como posible biomarcador del déficit motor dado que se demuestra que la reducción de AF en tronco el día 30 tiene una alta correlación con la evolución motora final

- Demostración de que los valores de AF del TCE eran significativamente menores en el lado afecto del infarto pero solo en el día 30 de evolución.

- Demostración de que el valor de corte ("cut-off") de la AF en el día 30 para discriminar un déficit motor era de 0.89.

Los resultados de esta tesis abren nuevas vías de trabajo e investigación como:

- Necesidad de estudios de validación y cualificación de los diferentes Biomarcadores del infarto cerebral en fase aguda y subaguda.

- Importancia del desarrollo de métodos automáticos y estandarizados de análisis del volumen del infarto.

La importancia y aplicabilidad de los resultados se concreta en varios puntos:

- La validación y cualificación de los biomarcadores puede mejorar y agilizar los futuros ensayos de tratamientos en fase aguda del infarto cerebral.

- Potencialidad de la Difusión tensor como marcador surrogado de la evolución clínica final en futuros ensayos clínicos en isquemia cerebral y en nuevos estrategias de rehabilitación.

- Apoyo para que los protocolos de RM de pacientes con infarto incluyan una secuencia de ITD.

- Apoyo para realización de control por RM a los 30 días de evolución incluyendo en el protocolo radiológico una secuencia de ITD.

5.4 Publicaciones y seguimiento

La tesis se presenta como compendio de 4 publicaciones realizadas que han tenido un alto impacto como se detalla en cada una de ellas:

1. Magnetic resonance imaging biomarkers of ischemic stroke: criteria for the validation of primary imaging biomarkers. Pedraza S, Puig J, Blasco G, Daunis-i-Estadella J,

Boada I, Bardera A, Prats A, Castellanos M, Serena J. Drug News Perspect. 2009 Oct; 22(8):481-6. IF de 2.598.

2. Reliability of the ABC/2 Method in Determining Acute Infarct Volume. Pedraza S, Puig J, Blasco G, Daunis-I-Estadella J, Boada I, Bardera A, Castellanos M, Serena J. J Neuroimaging. 2011 Mar 29. IG 1.287.

3. Acute Damage to the Posterior Limb of the Internal Capsule on Diffusion Tensor Tractography as an Early Imaging Predictor of Motor Outcome after Stroke. Puig J, Pedraza S, Blasco G, Daunis-I-Estadella J, Prados F, Remollo S, Prats A, Soria G, Boada I, Castellanos M, Serena J. AJNR Am J Neuroradiol. 2011, Ap 7, 32:857-63. IF: 3.464.

4. Wallerian Degeneration in the Corticospinal Tract Evaluated by Diffusion Tensor Imaging Correlates with Motor Deficit 30 Days after Middle Cerebral Artery Ischemic Stroke. Puig J, Pedraza S, Blasco G, Daunis-I-Estadella J, Prats A, Prados F, Boada I, Castellanos M, Sánchez-González J, Remollo S, Laguillo G, Quiles AM, Gómez E, Serena J. AJNR Am J Neuroradiol. 2010 Mar 18. 1324-30. IF: 3.464.

Las publicaciones de esta tesis han sido la base para la realización de los siguientes proyectos de investigación financiados:

- Proyecto "WAKEUP. Efficacy and safety of MRI-based thrombolysis in wake-up stroke: a randomised, double-blind, placebo-controlled trial) Agency "European Union-VII programme" REF: No 278276. 2011/2016. Principal Investigador general: Prof. Dr.

Christian Gerloff. Salvador Pedraza es el Investigador principal en España y director del comité de análisis de estudios radiológicos.

- Proyecto Desarrollo de un biomarcador radiológico basado en el análisis de la conectividad estructuras y funcional en pacientes con infarto cerebral agudo para la predicción de la recuperación funcional. Entidad financiadora: "BECA FIS" REF PI13/02545. 2013-2016. Salvador Pedraza es el Investigador principal.

Es importante destacar la relación con la tesis de Josep Puig Alcántara que analiza específicamente la utilidad de la DTI en el infarto cerebral para determinar el tiempo de evolución y la capacidad predictiva a largo plazo.

7. Conclusiones

Esta tesis realiza aportaciones importantes como:

- Propuesta pionera de un marco conceptual sobre los criterios de validez de un Biomarcadores de imagen por RM en el infarto cerebral con una revisión crítica de la validez de un panel de 8 biomarcadores y con propuesta de los campos concretos a mejorar en cada uno de ellos.

- Demostración de que la cuantificación del volumen del infarto con el método ABC/2 muestra una buena correlación con el método planimétrico y posee una buena reproducibilidad pero tiene un valor clínico limitado dado que exagera el volumen real del infarto cerebral.

- Demostración de que la afectación del TCE a nivel del brazo posterior de la capsula interna es un biomarcador predictivo de una mala evolución motora en un paciente con infarto cerebral.

- Demostración de la reducción de la AF del TCE como biomarcador de la existencia de degeneración walleriana es un buen biomarcador predictivo del déficit motor en el día 30 de evolución del paciente con infarto cerebral.

Los resultados de esta tesis abren nuevas vías de trabajo e investigación como:

- Necesidad de estudios detallados de validación y cualificación de cada uno de los diferentes Biomarcadores del infarto cerebral en fase aguda y subaguda.

- Importancia del desarrollo de métodos automáticos y estandarizados para cuantificación del volumen del infarto en fase aguda.

La importancia y aplicabilidad de los resultados se concreta en varios puntos:

- La validación y cualificación de los biomarcadores radiológicos puede mejorar y agilizar los futuros ensayos de tratamientos en fase aguda del infarto cerebral
- Potencialidad de la Difusión tensor como marcador surrogado de la evolución clínica final en futuros proyectos de investigación en isquemia cerebral

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