Study in Novel Neuro-muscular Imaging Biomarkers for Motor Outcome in Stroke

(SINONIMS Study)

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List of abbreviations

AD ……Axial Diffusivity

CAV ……Centre for Aging and Vitality, Newcastle University

CST ……Cortico-spinal Tract

CRF ……Case Report Form

CTO ……Clinical Trial Officer

DTI ……..Diffusion Tensor Imaging

FA ……Fractional Anisotropy

MD ……..Mean Diffusivity

MRI ……..Magnetic Resonance Imaging

NIHSS ……..National Institute of Health Stroke Score

PI ……..Principal Investigator

RA ……Research Associate

RD ……Radial Diffusivity

ROI ……...Region of Interest

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Summary

A common consequence of ischaemic stroke is the loss of limb function. This can lead to a stoke survivor being unable to continue to live an independent life. It would be beneficial to stroke survivors if clinicians could accurately predict recovery of limb function. An accurate prognosis would also enable treatment plans such as the content and duration of rehabilitation to be optimised. However, at present there is a lack of reliable methods for predicting recovery of movement after stroke.

Advanced magnetic resonance imaging (MRI) imaging can demonstrate the effect of stroke on the nerve pathways in the brain and the changes in the affected muscles in great detail. Advanced MRI has shown early promise in making recovery predictions in small-scale studies. Our research aims to build on this evidence and answer the question of whether advanced MRI can be used to predict how well stroke survivors will recover lower limb function.

We aim to study 30 participants who have had a first ever ischaemic stroke within the previous one to four weeks causing different degrees of stroke severity affecting the lower limb. They will undergo MRI brain and lower limb muscle scans lasting 40-45mins in a dedicated MRI facility in Newcastle University's Campus for Ageing and Vitality or in the Neuroradiology department, Royal Victoria Infirmary, Newcastle-upon-Tyne. A test of physical strength and functional ability of the affected leg lasting about 30mins will be done by a member of the research team around the time of the MRI scan and repeated 3 months after stroke. Analysis of our findings will determine whether advanced MRI scan findings are linked to the stroke survivors' recovery of limb function.

Background information

Stroke is one of the most important contributors to loss of independence, frailty and multi-morbidity in the elderly. Long-term motor impairment is the most frequent consequence of stroke with 50% of patients suffering from hemiparesis and 30% being unable to walk without assistance (1). Appropriate and targeted rehabilitation is the key in improving the outcome of stroke survivors. This is currently hampered by the fact that there is no accurate or widely accepted early prognostic tool to predict stroke recovery. A number of attempts have been made to use advanced imaging with or without clinical parameters to develop accurate biomarkers for recovery. For example the PREP algorithm for upper limb recovery uses a combination of clinical (SAFE or Shoulder Abduction Finger Extension score), neurophysiological (TMS or Transcranial Magnetic Stimulation) and imaging biomarkers (DTI or Diffusion Tensor Imaging) to predict upper limb outcome (2) with moderate accuracy. However there is no such tool available for lower limb motor recovery, even though improved mobility is key to a stroke survivor being able to live independently.

Eighty percent of strokes are caused by cerebral infarction, also called ischaemic strokes. After ischaemic stroke in particular, there are a number of micro-structural effects on the brain such as disconnection of white matter pathways, dieback of these pathways distant from the site of damage (i.e. Wallerian degeneration) and loss of functional networks. Advanced MRI techniques can now measure these effects on brain tissue in detail. Most of the available evidence centres on integrity of cortico-spinal tracts as measured by diffusion tensor imaging (DTI). Restriction to water diffusion by the interaction with the cell structure means diffusion is higher along the direction of an axon/tract than across it. This orientational restriction is quantified by the degree of diffusion anisotropy. Loss of integrity of white matter pathways due to Wallerian degeneration after stroke causes change in anisotropy which is measured by certain MRI metrics, the commonest of which is fractional anisotropy (FA). A good correlation was demonstrated between fractional anisotropy in pons 30 days after stroke and subsequent motor outcome in a single-centre observational study (3). Therefore the advancement of imaging techniques raises the possibility that radiological data could now act as a biomarker for outcome, but the ideal combination of information is unclear.

Although brain is the primary organ affected in stroke, skeletal muscle is the main end-organ accountable for motor disability. Sarcopenia or loss of quality and quantity of muscle associated with increasing age and is an important contributor to frailty and multi-morbidity in this population. Stroke is also an important contributor to muscle condition and mass, which interacts with sarcopenia and in combination they both impact upon the recovery process and the outcome of patients. Greater understanding of the structural, metabolic and functional characteristics of sarcopenia in a disease-specific setting such as stroke, could enhance our understanding of sarcopenia in general as well as stroke recovery.

While a lot of attention has been paid to the brain injury and neurological consequences of stroke, relatively less research has focussed upon the structural, metabolic and functional aspect of the affected muscles. Stroke-related sarcopenia is not recognised in current guidelines for stroke

therapy and rehabilitation. According to a systematic review, the evidence base for stroke-related sarcopenia comprises of 490 patients from 14 studies (including one UK study), which is miniscule compared to the body of evidence available for stroke in general (4). Only one of the studies included MRI imaging, where it was used to measure cross-sectional area of the arm (5). To our knowledge there is no MRI study which looks at lower limb muscle quality and quantity in early post-stroke setting.

Structural adaptive changes in muscle tissue have been observed to start as early as four hours after cerebral infarction which may be related to disrupted synaptic transmission of muscle innervating motor neurons which then leads to a reduction in motor units (6). A number of mechanisms influence the quantity and quality of the muscle tissue affected by stroke e.g. re-innervation, fibre-type shift, disuse atrophy and local inflammation (7). This inter-individual variability of end-organ changes in skeletal muscles, some of which may reflect other processes pre-stroke, could contribute towards the variability in outcomes after rehabilitation. Therefore a combined study of connectivity changes in brain after stroke together with changes in affected muscles may result in a prognostic tool for motor outcome and ultimately help individualise rehabilitation therapy e.g. neurological recovery may require greater cognitive engagement during rehabilitation activities or repair of interrupted pathways with stem cell therapy, whereas a patient with indicators for a relatively poor “muscle prognosis” may benefit from more intensive exercise practice to improve conditioning. Newcastle University is at the forefront of imaging of skeletal muscles and there is significant interest and expertise in qualitative and quantitative methodologies of assessing skeletal muscles in different disease processes (8). The ability of multi-modality MRI to assess the underlying pathophysiological process in sarcopenia has also been established by other research groups (9).

In summary, stroke-related sarcopenia and lower limb motor recovery are relatively under-researched areas in post-stroke setting. In addition, despite promising early imaging evidence from imaging studies, accurate early biomarkers of stroke recovery have not been fully developed and evaluated. The purpose of this research is to address the gap in research by: a) using advanced imaging to study structural, metabolic and functional characteristics of lower limb muscles in post-stroke setting b) addressing the translational gap between early evidence of MRI biomarkers for motor recovery in brain and muscles and their potential use after ischaemic stroke by developing biomarkers for predicting lower limb motor recovery and c) widening our understanding of sarcopenia in general and thereby help prevent frailty and multi-morbidity in elderly population.

Aim

To study the association and interaction between motor outcomes and advanced MRI biomarkers in the brain and affected lower limb muscles after first ever ischaemic stroke.

Objectives

- To describe the relationship between stroke-related sarcopenia with loss of connectivity in the brain

- To describe the relative strengths of correlation of individual muscle and brain imaging biomarkers to motor outcome (as determined by lower limb Fugl-Meyer score, lower limb motor NIHSS and dynamometry measurement) compared to that of a combination of imaging biomarkers to motor outcome

- To develop a predictive model of motor outcome at 3 months based on brain and muscle biomarkers obtained 1-4 weeks after stroke

- To establish the feasibility of obtaining simultaneous brain and muscle MRI imaging relatively early after stroke by description of recruitment rate, drop-out rate and rate of incomplete and sub-optimal imaging

- To obtain data to inform the design and sample size required for a blinded observational study to confirm the operational characteristics of an algorithm using baseline advanced MRI biomarkers of neurological injury and sarcopenia to predict motor outcome.

Study setting

Potential participants will be identified and recruited from two NHS stroke services in North East England. Patients will undergo a MRI study at 1-4 weeks following onset of symptoms of stroke. The MRI study will be carried out in Magnetic Resonance Centre at the Campus of Aging and Vitality, Newcastle University or in the Neuroradiology department, Royal Victoria Infirmary, Newcastle-upon-Tyne.

Study population

Inclusion criteria:

a) Adults aged 18 or over with first ever unilateral supra-tentorial ischaemic stroke.
b) Unilateral lower limb motor deficit +/- upper limb motor deficit.
c) Less than 4 weeks after stroke onset.

Exclusion criteria:

a) Absolute contra-indication to MRI (e.g. pacemaker).
b) ~~Posterior circulation~~ Cerebellar stroke or haemorrhagic stroke (haemorrhagic transformation of ischaemic stroke is not considered as an exclusion criterion).
c) Previous history of anterior circulation stroke (clinically or radiologically) or posterior circulation stroke with residual clinical deficit.
d) Lack of capacity to provide informed consent.
e) ~~Unable to transfer independently or with assistance of one person if scanned in Newcastle University or with assistance of two people if scanned in Royal Victoria Infirmary.~~ Unable to transfer with the assistance of two people for patients scanned in Newcastle University.
f) Unable to answer MRI safety questionnaire.

g) Moderate to high level of dependency prior to stroke (modified Rankin score of >2).
h) Any other pre-existing co-morbidity causing a significant lower limb deficit.
~~i) Hip or knee replacement of either lower limb.~~

NB. Patients requiring a hoist for transfer will not be considered a contra-indication if scanned in RVI although it will be a contra-indication for patients scanned in Newcastle University.

Case ascertainment, consent and recruitment

Appendix 1 summarises study processes.

Eligible patients will first be identified in hospital by NIHR CRN Clinical Trial Officers (CTO) providing NHS research support or other member(s) of the clinical team caring for the patient. Potential participants will be approached for discussion about the study and provided with a patient information leaflet. After allowing sufficient time for this information to be considered (>24 hours), a member of the research staff will ascertain whether the patient wishes to take part in the study. If so, the patient will provide written consent for their contact information to be passed to the study research associate at Newcastle University.

The study research associate will provide a more detailed explanation of the programme and subsequently obtain full written informed consent. The original consent form will be retained in the study site file. A copy of the consent form will be filed in the medical notes and a copy given to the participant. Due to the nature of this study and its small size, we plan for the information sheet and consent form to be available only in English. However, interpreters and translation of written material will be considered should a potentially eligible patient require this. If a patient has clearly indicated his/her consent but is unable to sign a consent form due to a disability, then a witness will be able to sign the consent form on their behalf.

Patients will be given the choice to participate in this study in addition to being part of another research study. If the patient agrees then co-enrolment will be allowed.

As this is an early observational study, a formal sample size calculation has not been performed. We aim to recruit 30 patients to reflect a range of walking abilities after stroke. In order to ensure that a range of stroke severity is represented in the study population, attempts will be made to ensure that one group of patients will not be over-represented. As mild patients (lower limb NIHSS of 1 or 2) are likely to be more easy to recruit, attempts will be made to ensure that the study includes moderate to severe category of stroke patients as well.

Recruitment will cease once 30 patients have attended for MRI studies. If a patient attends for but is unable to tolerate/complete the MRI study then he/she will continue to be in the study and be part of the feasibility analysis. If a patient is enrolled in the study but does not attend the MRI study, then the clinical assessment (s) if undertaken will be reported. Such a patient will be replaced by

another patient willing to undergo MRI so that the total number of 30 patients attending for MRI is achieved.

Data collection

After informed consent is obtained the following baseline demographic and clinical data will be recorded:

- Patient demographics (age, sex; handedness)
- Date of onset of stroke symptoms
- Pre-morbid modified Rankin score
- Pre-morbid walking status (independent or not)
- Associated co-morbidities e.g. presence of diabetes, hypertension, arthritis of affected limb.
- Stroke treatment (thrombolysis/thrombectomy).

Following this, two structured clinical assessments will be undertaken as follows:

Baseline clinical assessment:

Clinical assessment will be completed by a trained observer who will be blinded to the MRI data. This will take approx. 30 mins. It will be undertaken on the day of the MRI study if possible to minimise the number of times the patient has to travel. If this is not feasible or if the patient is unable to have both imaging and clinical assessment on the same day due to their physical condition, then this will be arranged within a week of the MRI study. If done on the same day, the clinical assessment will be undertaken at a dedicated facility at the Magnetic Resonance Centre, Newcastle University, or in the Department of Stroke Medicine, Ward 41, Royal Victoria Infirmary, Newcastle-upon-Tyne.

Following data will be collected during the baseline clinical assessment:

- Lower limb Fugl-Meyer score (Appendix 2, 10min)

- Lower limb motor NIHSS score (Appendix 3, 10min)
- Lower limb dynamometry (Appendix 4, 5min)
- Functional ambulatory score (Appendix 5, 5min).

Follow-up clinical assessment

A single follow up assessment of motor recovery will take place in Magnetic Resonance Centre in Newcastle University. This will take place three months +/- 7 days after the onset of stroke. The follow-up period can be extended to 6 months for a limited number of patients under exceptional circumstances. The following assessments will be undertaken:

- Lower limb Fugl-Meyer score (10min)
- Lower limb motor NIHSS score (10min)
- Lower limb dynamometry (5min)
- Functional ambulatory score (5min).

The study timelines are detailed in Appendix 6.

Planned advanced MRI imaging

A 3 Tesla MRI study will take place in the Magnetic Resonance Imaging Centre in Centre for Aging and Vitality (CAV) in Newcastle University or in the Neuroradiology department, Royal Victoria Infirmary, Newcastle-upon-Tyne. The research team will liaise with the participant (or the relevant clinical team if still in hospital) and the MRI team to pre-book an appointment. The study will take approximately 45-50 min. Travel and parking costs for the participants will be re-imbursed from the study budget.

The CTO/member of the clinical team at the recruiting site will undertake an initial MRI safety screening (for absolute contraindications e.g. cardiac pacemaker) before obtaining permission to pass on the patient's details to the research associate. A member of the research team will subsequently undertake additional screening for MRI safety at the time of booking the MRI study. A member of the MRI team will undertake a final MRI safety screening with the patient on the day immediately before the study is undertaken. Any instance of study being cancelled due to MRI safety concern will be documented and reported alongside study results.

The following MRI sequences will be performed (Appendix 7):

1. Brain imaging: Structural - 3D volume T1; Functional imaging: DTI (approx. 15 min).

2. Muscle imaging: 3D volume T1, STIR, 3 point Dixon or equivalent, MR spectroscopy of the thigh muscles (approx. 25-30 min).

The images will be transferred to a workstation for review by an observer who will be blinded to the clinical status of the patient. Following assessments will be made on the structural brain imaging: lesion location, lesion side, lesion number, lesion volume, anatomical structures involved, vascular territory involved, any other pathology.

The following quantitative assessments will be made from the functional brain imaging sequences: DTI imaging – a region of interest will be drawn on the pyramidal tracts at the level of mid-brain and internal capsule and the following parameter will be measured: FA, relative FA (i.e. FA ratio between normal and abnormal sides) and axial, radial and mean diffusivity.

The following assessments will be made from the muscle MRI:

a) Muscle volume, based on a 6 point scale described by Mercuri et al (10)

b) Muscle oedema, based on a 4 point scale (absent, mild, moderate, severe)

c) Fatty infiltration, based on 3 point Dixon method or equivalent (8)

d) Intra-myocellular lipid content on MR spectroscopy.

Image data

The following data will be extracted from the cranial MRI studies:

- Site of infarction – location will be anatomically classified as frontal, parietal, temporal, occipital, basal ganglia; it will also be classified along vascular territories e.g. middle cerebral artery, anterior cerebral artery or posterior cerebral artery, etc
- Side of infarction
- Infarct volume – region of interest (ROI) will be placed on the affected region and volume will be measured using validated software
- Cross-sectional DTI measurements - axial, radial and mean diffusivity (AD, RD and MD), fractional anisotropy (FA) (ROIs will be placed in the cortico-spinal tracts on both sides at the levels of corona radiata, internal capsule and cerebral peduncle).
- Whole tract DTI measurement – 3D Tractographic analysis of the whole cortico-spinal tract on both sides will be made with measurements of whole tract volume, MD and FA.

The following data will be extracted from the muscle MRI studies:

- Muscle volume – this will be performed on the T1 volume data based on a 6 point scale described by Mercuri et al. The volume of the whole thigh and that of the largest thigh muscle (vastus lateralis) will be measured.

- Fat fraction analysis – this will be done using the fat only images obtained from 3 point Dixon method.

- Intra-myocellular lipid content – MR spectroscopy will be performed on the Vastus Lateralis muscle on both sides. Intra-myocellular lipid fraction will be obtained by interrogating the two lipid peaks seen on the MR spectrum.

- Muscle oedema – this will be obtained from the STIR sequence based on a 4 point scale (absent, mild, moderate, severe).

All measurements will be made in the affected and normal sides and a ratio of parameters between the two sides will be calculated.

Data storage

Paper CRFs will be transferred in a secure manner by the study RA from the recruiting site to Newcastle University. The original CRFs will be stored securely in a locked safe in the Stroke Research Centre at Newcastle University. The consent forms will be filed in the patient notes.

Clinical data will be entered into a database by the study RA which will then be stored securely on a Newcastle University server. Imaging data will be stored securely in the space allocated to Magnetic Resonance Centre in the Newcastle University data store. The research team will comply with the data protection requirements as set out in Good Medical Practice guidelines, by the study sponsor and the Data Protection Act.

Data analysis

In order to effectively explore this research question, two hypotheses are proposed:

Hypothesis A: Sarcopenia and loss of motor impairment will be most pronounced in those patients whose ischaemic stroke causes greatest damage to the cortico-spinal (CS) tract and motor pathways as determined by advanced neuroimaging.

Hypothesis B: A combination of imaging biomarkers of loss of connectivity in brain and sarcopenia will increase the ability to predict motor outcome in stroke when compared to individual biomarkers alone.

In line with the study hypotheses the following broad categories of analyses will be undertaken.

Analysis A: Degree of sarcopenia (as determined by study of muscle volume, fat fraction and oedema on MRI) will be used as a covariate in a regression analysis between motor outcome (lower limb Fugl-Meyer score) and neuroimaging assessment of CS tract integrity (fractional anisotropy or FA as determined from diffusion tensor imaging or DTI).

Analysis B: Correlation of a combined biomarker of brain connectivity and sarcopenia versus motor outcome (as determined by lower limb Fugl-Meyer score) will be stronger than correlation between individual biomarkers and motor outcome.

The following individual statistical analyses will be performed:

- Correlation between cortico-spinal tract (CST) DTI data (AD, RD, MD and FA of cross sectional data and MD and FA of 3D tractographic data) and muscle MRI data (volume, fat fraction, intra-myocellular lipid fraction and muscle oedema)
- Correlation between CST DTI data and lower limb Fugl-Meyer score, motor NIHSS and dynamometry measurement at 3 months
- Correlation between CST DTI data and change in Fugl-Meyer score, motor NIHSS and dynamometry measurement between baseline and 3 months
- Correlation between muscle MRI data and Fugl-Meyer, motor NIHSS and dynamometry measurement score at 3 months
- Correlation between muscle MRI data and change in Fugl-Meyer score, motor NIHSS and dynamometry measurement between baseline and 3 months
- Correlation between combined CST DTI/muscle MRI data and lower limb Fugl-Meyer score at 3 months
- Correlation between combined CST DTI/muscle MRI data and change in lower limb Fugl-Meyer score between baseline and 3 months
- Multi-variate regression analysis of muscle and cranial DTI data and lower limb Fugl-Meyer scores.

Study withdrawal

No specific study withdrawal criteria have been set. Participants may withdraw from the study at any time for any reason. Should a patient decide to withdraw from the study, a reason for withdrawal will be sought but patients can choose to withdraw without providing an explanation. If a participant decides to withdraw it will not affect the normal care they receive. Data collected prior to withdrawal will be used in the study analysis unless consent for this is specifically withdrawn.

Clinical teams, investigators or other members of research team may also withdraw participants from the study at any time if they feel it is no longer in their interest to continue, for example, because of inter-current illness.

Safety

MRI is already used in clinical practice with known risks which are minimised by a detailed MRI safety screening process. All study participants will be routinely screened with a standard MRI safety questionnaire before undergoing the study as detailed before (Please see Planned Advance MRI section). All patients with a contra-indication to MRI will be excluded from the study. The MRI study does not involve injection of any contrast agent.

As this is a blinded observational study without any change in patient care, standard adverse event reporting will not be completed.

Should a medical event occur which is serious and unexpected (results in death; is life-threatening; requires inpatient hospitalisation or prolongation of existing hospitalisation; results in persistent or significant disability or incapacity; consists of a congenital anomaly or birth defect; otherwise considered significant by investigator) and is perceived to be possibly or definitely related to a study intervention (for e.g. MRI imaging), a separate study Serious Adverse Event form will be completed. All such events will be reported to the principal investigator, study sponsor and Research Ethics Committee. Checking for SAE will take place at the time of the baseline and 3 month assessments.

All the MRI studies undertaken as part of the study will be reviewed by a Consultant Radiologist. If there is a significant unexpected finding on MRI, the PI will be informed who will then communicate to this to the hospital team, the GP and the patient on a case by case basis.

All deaths of study participants will be recorded.

Ethics and regulatory issues

The study sponsor will be Newcastle upon Tyne NHS Foundation Trust. The study will be conducted in accordance with Research Governance Framework for Health and Social Care Approval from NRES and local NHS R&D will be obtained in writing prior to the commencement of the study.

Confidentiality

Personal data will be regarded as strictly confidential. The study will comply with the new GDPR regulations. Records will be kept securely at Newcastle University with restricted access. The imaging data will be anonymised before analysis is undertaken. Patient identifiable information will be retained for up to 12 months following conclusion of the study. All other research documentation

will be retained for future audit and inspection for 10 years in line with sponsor policies. Participants will not be identified in any report or publication arising from this research.

Dissemination

Update on progress of the study will be provided to the research participants and their family through 6 monthly newsletters. At the end of the study, research participants will be invited to a presentation where the findings will be discussed with them. Results of the study will be published in a peer reviewed open access journal for wide dissemination.

Funding

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Appendix 1: Study processes

See attachment.

Appendix 2: Lower limb Fugl-Meyer score

See attachment.

Appendix 3: Lower limb motor NIHSS

The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.

0 = **No drift;** leg holds 30-degree position for full 5 seconds.

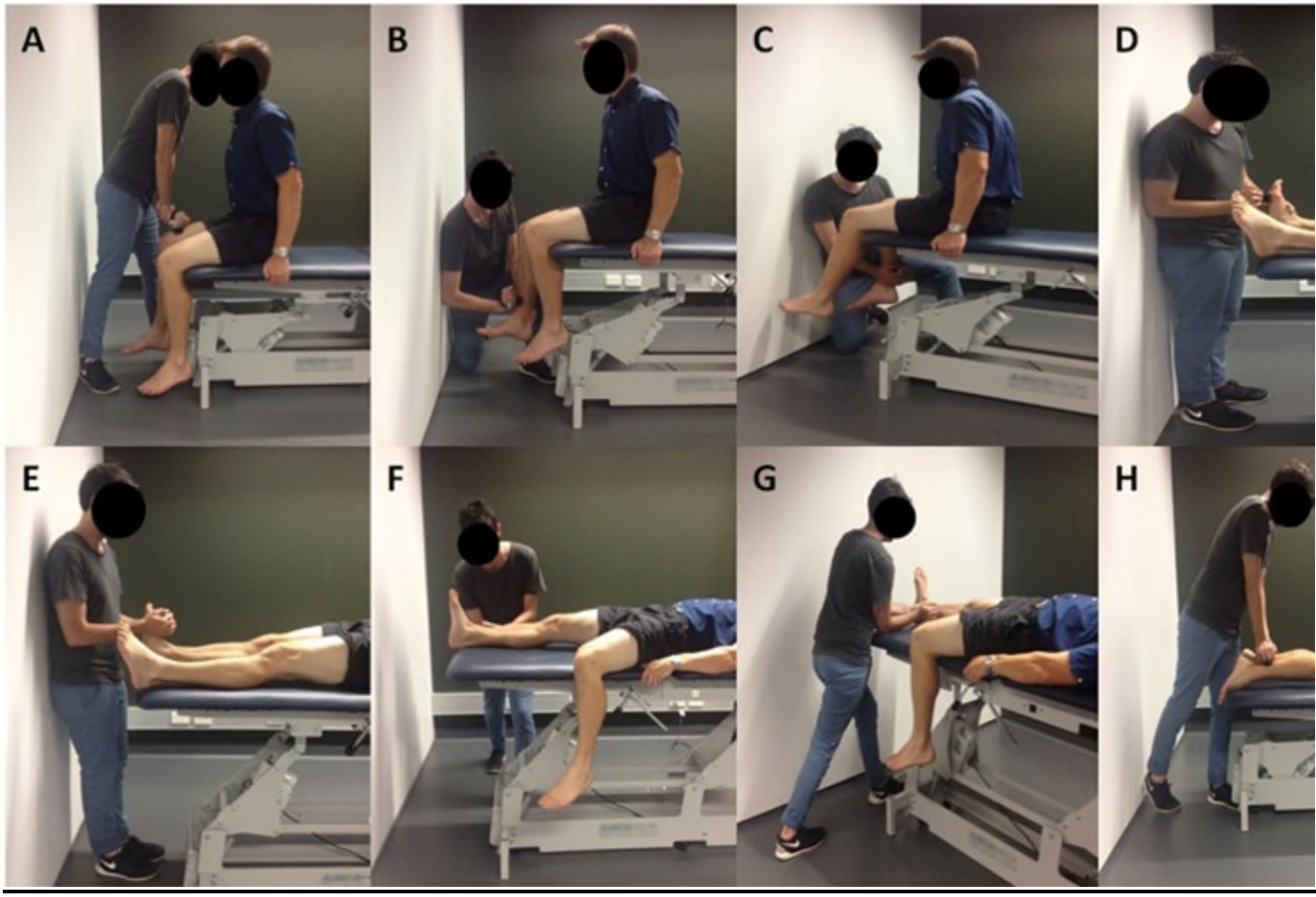
1 = **Drift;** leg falls by the end of the 5-second period but does not hit bed.

2 = **Some effort against gravity;** leg falls to bed by 5 seconds, but has some effort against gravity.

3 = **No effort against gravity;** leg falls to bed immediately.

4 = **No movement.**

Appendix 4: Lower limb dynamometry (Figures as per ref 11)



Testing positions for strength and power assessment.

Note: Same positions were used on the fixed dynamometer. (A) Hip flexors with the participant seated and hips and knees flexed at 90°. Dynamometer placed on the anterior aspect of the thigh, proximal to the knee joint. (B) Knee extensors with the participant seated and hip and knees flexed at 90°. Dynamometer placed on the anterior aspect of the shank, proximal to the ankle joint. (C) Knee flexors with the participant seated and hips and knees flexed at 90°. Dynamometer placed on the posterior aspect of the shank, proximal to the ankle joint. (D) Ankle plantarflexors with the participant lying supine with the ankle in plantargrade and hips and knees extended. Dynamometer placed over the metatarsal heads on the sole of the foot. (E) Ankle dorsiflexors with the participant

lying supine with the ankle relaxed and hips and knees extended. Dynamometer placed over the metatarsal heads on the dorsum of the foot. (F) Hip abductors with the participant lying supine and hips and knees extended. Dynamometer placed on the lateral aspect of the shank, proximal to the ankle joint. (G) Hip adductors with the participant lying supine and hips and knees extended. Dynamometer placed on the medial aspect of the shank, proximal to the ankle joint. (H) Hip extensors with the participant lying prone and hips and knees extended. Dynamometer placed on the posterior aspect of the shank, proximal to the ankle joint.

(The equipment and training will be provided by Stroke Research Group, Newcastle University).

Appendix 5: Functional ambulation categories

Scoring and Score Interpretation

Score	Category	Interpretation
0	Non-functional ambulator	
1	Ambulator, dependent on physical assistance – level I	Indicates a patient who requires continuous manual contact to support body weight as well as to maintain balance or to assist coordination.
2	Ambulator, dependent on physical assistance – level II	Indicates a patient who requires intermittent or continuous light touch to assist balance or coordination.
3	Ambulator, dependent on supervision	Indicates a patient who can ambulate on level surface without manual contact of another person but requires standby guarding of one person either for safety or verbal cueing.
4	Ambulator, independent level surface only	Indicates a patient who can ambulate independently on level surface but requires supervision to negotiate (e.g. stairs, inclines, non-level surfaces).
5	Ambulator, independent	Indicates a patient who can walk everywhere independently, including stairs.

Appendix 6: Study timelines

1	Complete study protocol	30/06/2018
2	Complete ethics and regulatory approval	31/12/2018
3	Appoint study RA	31/12/2018
4	Start patient recruitment	01/05/2019
5	Complete patient recruitment	31/10/2020
6	Complete follow-up	31/01/2021
7	Complete data cleansing, statistical analysis and report writing	01/06/2021

Appendix 7: MRI protocol

MR investigations will be performed at the Newcastle University MR Centre on a 3T Philips Achieva scanner or at the Royal Victoria Infirmary Neuroradiology department 3T Siemens Skyra scanner. Patients will have scans in two body areas, requiring them to be repositioned between the investigations. Brain imaging will use a standard 8 channel headcoil, while thigh imaging will use an 8 channel body receiver array coil. Imaging investigations are as detailed below:

BRAIN IMAGING:

1. **Scout**

Three axis scout image for patient localisation. **Acquisition time 1.00 mins.**

2. **Structural scan (T1w)**

Whole brain structural scan will be acquired using a 3D MPRAGE (magnetisation prepared gradient echo) scan with sagittal acquisition, slice thickness 1.0 mm, in plane resolution 1.0×1.0mm; TR = 8.3 ms; TE= 4.6 ms; flip angle = 8° ; SENSE factor = 2). **Acquisition time 4.40 mins**

3. **Diffusion imaging**

Diffusion tensor imaging acquisitions would be acquired using a 2D spin-echo, echo planar imaging diffusion-weighted sequence with 59 slices: TR=6100ms; TE=70ms; flip angle=90°; FOV=270×270mm, pixel size= 2.1×2.1mm; slice thickness = 2.1mm; Diffusion-weighting will be applied in 64 uniformly distributed directions (diffusion b=1000 $s.mm^{-2}$) including 6 acquisitions with no diffusion weighting (b=0 $s.mm^{-2}$).

An identical image with b=0 $s.mm^{-2}$ but with the phase encoding direction reversed will be collected for distortion correction purposes using the “TOP-UP” algorithm from the FSL software package which will be employed for analysis of the diffusion imaging data). **Acquisition time 7.30 mins**

Total duration: 15 mins

LEG IMAGING:

1. **Scout**

Three axis scout images for patient localisation. **Acquisition time 1 min.**

2. **Volumetric T1w**

T1w TSE sequence. TR/TE=633/20ms. 400×300mm FOV, 1.5mm in-plane resolution × 5 mm slice with 5 mm inter-slice gap (i.e. 10mm slice spacing) to cover 395mm extent. **Acquisition time 5.5 mins.**

3. **STIR**

Scan geometry to match T1w series, 400×300mm FOV, 1.5mm in-plane resolution × 5 mm slice with 5 mm inter-slice gap (i.e. 10mm slice spacing) to cover 395mm extent. TR/TE/TI=21000/30/190ms. **Acquisition time 11.5 mins.**

4. **3-point Dixon or equivalent**

Multi-TE acquisition with 3TEs specified for 3T. TR=100ms. 400×300 FOV, 2mm in-plane resolution × 10mm slice restricted sampled volume using 10 slices only. **Acquisition time 6 mins.**

5. **Proton MR Spectroscopy**

Single voxel STEAM MRS. Acquisition 5 mins per volume (estimated including shim and water suppression adjustment). **Acquisition time 10 mins.**

Total duration: 34 mins