-Study protocol

Title: Remote Ischaemic Conditioning for Fatigue After Stroke (RICFAST) – a pilot, single-blind, randomised, placebo controlled trial.

1. Project details

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1.3. Project title

Remote Ischaemic Conditioning for Fatigue After Stroke (RICFAST) – a pilot double-blind, randomised, placebo controlled trial.

1.4. STH Project Reference number

STH19508

1.5. Protocol version number and date

Version 2 09/07/2018

1.6. STH Directorate affiliation

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2. Research questions:

1- Do stroke patients undergoing rehabilitation find it acceptable to undertake chronic remote ischaemic conditioning (CRIC) for a period of 6 weeks?
2- Is it feasible to undertake a randomised control trial of CRIC to reduce fatigue and enhance the physical performance of patients undergoing rehabilitation following stroke?

3. Abstract

Background

Remote ischaemic conditioning (RIC) is a when ischaemia is induced to a limb for short periods of time by inflating pressure cuffs around arms or legs to above systolic pressures (mmHg). This procedure is performed for periods that avoid physical injury to the limbs, but induce neurohormonal, systemic or vascular changes in the body. These changes often result in improved collateralisation of blood supply to various areas of the body as well as improved efficiencies of cellular metabolism [Pell et al 1998]. This may have enhancing effects on physical abilities of patients undergoing rehabilitation after stroke, particularly when aiming to improve endurance and fatigue. Fatigue affects can affect up to 75% of patients who suffer a stroke [Wu et al 2014], which can be physical, cognitive or emotional, and can be a large barrier to progressing rehabilitation among such patients.

Method

In this pilot randomised controlled trial, a minimum of 34 patients who have suffered an ischeamic or haemorrhagic stroke who suffer from fatigue, will be recruited and randomised to receive either RIC or sham intervention protocols for 6 weeks (1 session of 4 cycles three times weekly). Each session will take approximately 40 minutes and will be administered by a post-graduate researcher.

Socio-demographic and clinical data will be collected at baseline by a clinical researcher at the assessment and rehabilitation centre (ARC), Nether Edge Hospital, Sheffield, or the clinical research facility (CRF) or the stroke unit (SU), Royal Hallamshire Hospital (RHH). Outcome measures including: fatigue measures (fatigue severity scale), duration of therapy undertaken (mins/week), peak oxygen consumption (mL/Kg/min), physical activity measures (activity monitor and questionnaire), Barthel Index (BI), PHQ9 and GAD 7 (measures of depression and anxiety), Modified Montreal Cognitive Assessment (MOCA, cognition), modified Rankin Score (mRS), and EQ-5D (measure of quality of life), will be assessed at baseline and at the end of the intervention period (week 6). Participants will undergo ECG analysis and have blood tests including full blood count, renal function, liver function, coagulation profile, and inflammatory markers, taken at baseline and at 6 weeks. These will be done in addition to routine care for the purposes of ensuring

participant safety and to review any changes RIC may cause. Healthcare utilisation and recurrence of vascular events will also be abstracted from electronic health records, including the number of hospital admissions, and new major vascular diagnoses. Follow up at 3, 6 and 12 months will collect data on Fatigue, PHQ-9, GAD, mRS, EQ-5D, recurrent vascular events and healthcare utilisation.

An electrocardiogram (ECG) will be performed on each participant at the beginning of the study and analysed for rate, rhythm, QT interval (QTc) and QRS morphology. Eight patients, randomly selected (4 CRIC arm and 4 sham) will also undergo MRI perfusion of the brain and brain spectroscopy at baseline and at week 6. This will evaluate changes detected in cerebral perfusion and in levels of tissue metabolites including: N-acetyl aspartate (that may indicated loss or damage to neuronal tissue), creatine (that may indicate cell death through ischaemia), and lactate (that may indicate anaerobic glycolysis) [Jansen 2006]. This will be done at the Royal Hallamshire Hospital. These participants will also have blood samples taken along with the other blood samples described that will be stored for future analysis (GLP-1, heat shock proteins, and micro-RNA analysis for pro-inflammatory and anti-inflammatory gene expression) with the patients consent. These samples will be sent to the Stoller Biomarker Discovery Centre (SBDC) in Manchester biochemical analysis.

A manual sphygmomanometer will be used to perform the conditioning protocol. The protocol will involve 4 x 5 minute cycles of arm or leg cuff inflation (to 200 mmHg of systolic blood pressure) followed by 5 minutes of deflation. The sham arm or leg will inflate cuff pressures to 10 mmHg, for the same number of cycles.

Acceptability of the intervention will be evaluated via the use of symptom diaries completed by patients throughout the intervention period that will aim to record any adverse events or unexpected symptoms, as well as acceptability measured using Likert scales. A clinical researcher will review participants 3 times weekly to provide support and review acceptability. Six participants will be invited to participate in semi-structured qualitative interviews with one of the study clinicians to allow an in-depth exploration of participant expectations and experiences of the treatment. This will take place during the last scheduled visit or at another mutually exclusive time between participant and research clinician.

Study feasibility will be determined by success criteria based on recruitment, outcome measure assessment, compliance with intervention and follow up (see primary outcomes section).

Results

Safety and success criteria - The main outcomes of this pilot RCT will be the safety, compliance, and acceptability of delivering a 6-week programme of CRIC intervention, and study feasibility. Safety will be defined as the absence of any serious adverse events (as defined by National Research Ethics Service – see safety section) related to CRIC. Compliance will be defined as the achievement of 80% of intended CRIC cycles, while acceptability of RIC will be defined as less than 1/3 of patients

reporting moderate or greater discomfort (scale 3 or 4 on Likert) associated with CRIC, as well as overall positive responses from qualitative interviews. Study feasibility will be defined as the ability to recruit to target (4 patients within the first 2 months), and completion of > 80% of baseline and follow up assessments.

Secondary outcomes will involve a comparison of changes in the outcome measures assessed at baseline and at 6 weeks, between CRIC and sham treatment arms to provide an estimate of effect size for informing the design / sample size of a future study. Continuous data will be compared using parametric or non-parametric analyses depending on whether data distribution is normal or not. Categorical data will be compared using Chi-square or one-way Anova. However, due to small sample size, appropriate power to detect significant differences for these measures will not be reached. This is primarily an exploratory study and the majority of data will be reported descriptively.

4. Originality

Remote ischaemic conditioning and its protective effects were discovered 30 years ago, in canine models of myocardial ischaemia [Murry et al 1986]. Since then its application has been tested for myocardial protection in elective [Cheung et al 2006] and emergent [Yellon et al 2015] coronary interventions, in animal and human models of cerebrovascular ischaemia [Hahn et al 2011, Hougaard et al 2014], and, more recently, to enhance physical performance in athletes [Tocco et al 2015, Patterson et al 2014]. This latter finding may be related to RIC induced changes in vasomotor responses that improve oxygen delivery to muscles [Duncker et al 1993] or improved preservation of endothelial function [Kharbanda et al 2001] during exercise-induced ischaemia. One small study investigated the use of RIC on exercise capacity in patients with heart failure [Mcdonald et al 2014] but no studies have yet investigated its use in patients undergoing rehabilitation after stroke. Further, no studies have evaluated the effects of RIC on fatigue, nor have any studies evaluated the effects of RIC on tissue metabolites in-vivo as examined by MRI brain perfusion and tissue spectroscopy.

5. Background

Remote ischaemic conditioning (RIC) is the phenomenon whereby brief episodes of reversible ischaemia are applied to a particular tissue or organ. This has been shown to be beneficial in the context of subsequent ischaemia of sufficient intensity to cause cell death. Since 1986, investigators have highlighted that RIC can reduce cardiac infarct size [Murray et al 1986], via release of ‘protective factors’ [Przyklenk et al 1993], that has seen its use extend to planned cardiac interventions [Sharma et al 2015] as well as acute stroke [Hahn et al 2011, Hougaard et al 2014]. A number of mechanisms for these effects have been proposed:

Neuronal pathway – an intact peripheral and autonomic neural circuit appears to be critical to modulate the effects of RIC as the use of ganglionic blockers (e.g. hexamethonium or trimetaphan) [Gho et al 1996, Loukogeorgakis et al 2005] or nerve

resection (peripheral and vagotomy) [Lim et al 2010, Brzozowksi et al 2004] inhibit these effects. Release of endogenous factors in the conditioned tissue such as adenosine and bradykinin are thought to stimulate C fibres in particular [Xiao et al 2001], that then medicate protection at a distant organ.

Humoral pathway – The presence of a blood-borne factor (or factors) that mediates the protective effects of RIC is suggested by two observations Firstly, blood from a conditioned organ or animal can exert its protective effects in a naïve recipient that is subsequently affected by ischaemia [Dickson et al 2001, McDonald et al 2014]. Secondly, following ischaemic conditioning, a period of reperfusion to an organ (e.g. the heart) is required for the protective effects to be observed [Weinbrenner et al 2002].

Systemic response - RIC in healthy controls has also been shown to modulate the immune response, with suppression of pro-inflammatory genes, such as those involved in leucocyte motility and function [Saxena et al 2010], as well as upregulation of anti-inflammatory genes encoding factors such as heat-shock-protein 70 and calplastin [Konstantinov et al 2004].

More recently however, the use of RIC to enhance physical performance in athletes has drawn great interest. To date there have been 8 randomised controlled trials (RCTs) investigating this in long distance running, sprinting, cycle ergometry and swimming [Sharma et al 2015]. All have used RIC protocols that are similar to those used in cardiac populations, with conflicting results: 5 revealed significant improvements in outcomes such as power output, time-trial performance, lower serum lactate levels, and maximal exercise performance; while 3 showed no added benefit over control or sham. It has been postulated that these effects are derived due to vasodilatory effects of RIC mediated by adenosine in the vascular bed, as well as effects on mitochondrial function that improve efficiency of oxygen utilisation within the musculature [Duncker et al 1993]. These properties may be useful if applied to patients suffering recent stroke who are undergoing rehabilitation. Up to 75% of patients experience fatigue following stroke [Wu et al 2014] that can be physical and cognitive. Stroke often results in impairments of physical or cognitive function that reduce the efficiency of activities, and can increase their oxygen consumption requirements by 50% [Walters et al 1989, Olney et al 1991]. Fatigue is often cited as a reason for limiting rehabilitation after stroke. The effects of RIC on improving blood and oxygen delivery to muscles, and on improving the efficiency of oxygen utilisation may help this group of patients and should be investigated.

Alignment of Strategic objectives of the directorate

The host institution is Sheffield Teaching Hospitals in collaboration with University of Sheffield. The NHS sponsor is an active participant in stroke trials and the University of Sheffield (affiliation of the Principal Investigators) is committed to the development of original research in stroke. Dr Ali is a consultant stroke physician, geriatrician, and senior honorary lecturer at the University of Sheffield. Dr Kirsty Harkness and Jessica Redgrave are consultant stroke physicians and senior honorary lecturers at the University of Sheffield. Both are committed to developing original

research studies in the area of stroke rehabilitation. Professor Arshad Majid is a professor of neurovascular science at the University of Sheffield and an honorary consultant in neurology specialising in stroke at Sheffield Teaching Hospitals. The trial will be conducted in compliance with the protocol, Good Clinical Practice, STH Standard Operating Procedures and applicable regulatory requirements. Bethany Moyles is a PhD student at the University of Sheffield who has experience in recruiting to stroke research trials. This research project also aligns with the strategic objectives of the Biomedical Research Centre (BRC) in developing studies evaluating mechanisms and effects of interventions that are likely to result directly in improved patient outcomes and experiences.

6. Plan of investigation

Aim

1- To assess if stroke patients undergoing rehabilitation find it acceptable to undertake chronic remote ischaemic conditioning (CRIC) for a period of 6 weeks?
2- To establish if it is feasible to undertake a randomised control trial of CRIC to reduce fatigue and enhance the physical performance of patients undergoing rehabilitation following stroke?

6.1. Methods Justification

We will aim to be as inclusive as possible for eligibility into the study, but will utilise exclusion criteria that have been used in prior trials of RIC in cardiac and stroke populations such as the presence of peripheral neuropathy, and cerebrovascular or cardiovascular hospitalisation within 4 weeks [Lavi et al 2014, Hougaard et al 2014]. The need for participants to be at least 6 weeks post stroke and discharged into the community reflects the preference for patient's neurological and cardiovascular status to have stabilised. Enough time should have elapsed from what may have been a severe ischaemic or haemorrhagic event that is likely to have induced a response similar to, if not more intense than RIC.

The outcome measures and scales that will be used in the study (e.g. fatigue severity score, PHQ-9, GAD, MOCA, EQ-5D, VO2 max, 6MWT) have all been validated in stroke populations [Mead et al 2007, Van Hout et al 2012, Blackburn et al 2013, Eng et al 2004].

We have used the fatigue severity scale-7 (FSS-7) for fatigue assessment [Krupp et al 1989]. This is a 7-item self-administered questionnaire that measures the effect of fatigue on a persons activities and lifestyle. Each item is scored on a 7-point scale with 1 = strongly agree and 7 = strongly disagree. The scores thus range from 7 to 49, with the greater the score indicating greater fatigue [Lerdal et al 2011]. There are population normative results [Valko et al 2008], and minimal clinically important

differences (MCID) published [Nordin et al 2016], and this fatigue scale is the most commonly studied in patients with stroke [Mead et al 2007].

The protocol for CRIC (ischaemia-reperfusion cycle duration of 5 minutes each, repeated 4 times with maximum pressures of 200 mmHg) is reflective of those used most commonly among prior cardiac and stroke studies that have resulted in positive responses with regard to physical performance measures (VO2 max) [Sharma et al 2015]. While a 'washout period' for the neurohumoral effects of RIC has been estimated at 7 days [Housenloy et al 2010] we have decided not to pursue a cross-over design, as this remains controversial. Further, while we will aim to for participants to complete 5 minutes of the intervention inflation cycles, we will be evaluating the acceptability of the intervention, and thus we will allow participants to tolerate as long a duration of inflation as they can and record this during the follow up visits. If participants cannot tolerate arm conditioning they will be offered leg conditioning as this study aims to assess acceptability of the treatment.

We will use a sham intervention in patients randomised to the control group that involves inflation of the limb cuff to 20mmHg. This has been chosen as it is the most common sham protocol used in prior studies. It is recognised that it may be difficult to ensure participant blinding due to the pressure differences with cuff inflations. However, care will be taken to ensure the patient information leaflet will describe the intervention as an inflation of the cuff, without mention of the pressures that would be expected. The sham CRIC will be designed to make the same noises that the true CRIC intervention will make and for the same length of time. Outcome assessments will be blinded to randomisation as they will be undertaken by a researcher not known to the participants before and who will not ask about the intervention during the assessments. An online randomisation system will be used rather than sealed envelope randomisation.

We will document adverse events using standard NRES definitions (www.hra.nhs.uk). For example, serious adverse events (SAE) will include events that result in death, are life-threatening, require hospitalisation or result in persistent or significant disability, or is a congenital anomaly or birth defect. There are no agreements or guidelines on what is considered an acceptable rate of adverse events as this is dependent on the study population, comorbidities etc. Adverse events need to be scrutinised to determine whether they are likely to be related to the intervention itself. For the purposes of this pilot RCT (n=34) we will define safety as there being no serious adverse events relating to CRIC and less than 10 adverse events in total across all 34 participants.

Six participants recruited to the study will also undertake qualitative semi-structured interviews using topic guides with a researcher trained in thematic analysis to gain insight into their experiences with CRIC. This will help evaluate the acceptability of CRIC by asking about both positive and negative experiences, as well as explore unanticipated effects of the treatment. The researcher will also enquire as to the barriers and facilitators to successful recruitment and retention to the study, which will help with future clinical trials and the potential implementation of CRIC into clinical practice.

The use of MRI perfusion of the brain and tissue spectroscopy to evaluate any effects of CRIC on tissue composition is novel. We still do not have complete understanding of how RIC exerts its effects, particularly in humans, and as such this study will provide an opportunity to further evaluate this. If RIC causes a cascade of effects that result in improved tissue perfusion or efficiency in oxygen metabolism then we may see changes in cerebral perfusion and / or the metabolites detected in the brain (spectroscopy). Prof Iain Wilkinson will facilitate scanning and interpretation of scans.

6.2. Design

Pilot, double blind, randomised, placebo controlled trial within a single centre.

6.3. Setting

Participants will have baseline and subsequent clinical assessments, at the ARC, Nether Edge Hospital, Sheffield or the Clinical Research Facility or the Stroke Unit at the RHH.

6.4. Participants:

A minimum of 34 participants will be recruited from the community stroke services over 24 months from August 2018 to August 2020 (GANTT chart). All participants will have supervised CRIC delivered by a clinical researcher at scheduled study visits to ARC.

Inclusion criteria:

- Adults (aged > 18 years) who have had an ischaemic or haemorrhagic stroke at least 6 weeks prior.
- Symptoms of debilitating fatigue for at least 4 weeks (fatigues severity score of 28 or more).

Exclusion criteria:

- History or presence of significant peripheral vascular disease in the upper limbs.
- History or presence of complex neuropathic pains or peripheral neuropathy in the arms.
- Presence of lymphoedema in the arms.
- Presence of skin ulceration to the arms.
- Hospitalisation for cardiovascular or cerebrovascular disease within the last 4 weeks.
- Uncontrolled arrhythmia, hypertension, diabetes or angina.

- Third degree heart block or progressive heart failure.
- Acute aortic dissection, myocarditis, or pericarditis.
- Acute deep vein thrombosis, pulmonary embolism or pulmonary infection.
- Suspected or known dissecting aneurysm.
- Uncontrolled visual or vestibular disturbance.
- Known or suspected cause of fatigue e.g. obstructive sleep apnoea (Epworth > 15), depression (PHQ-9 > 14).
- Modified Rankin Score > 4.

6.5. Sample Size

The sample size of at least 34 has been chosen based on the number of participants evaluated in studies of physical performance in athletes (range 11 – 25) that reported outcome measures based on exercise performance e.g. running speed, peak and mean power outputs, trial time performances, oxygen consumption. Further, a cross-over RCT of RIC to improve peak oxygen consumption in heart failure included 20 patients [McDonald et al 2014]. A sample size of 30 is also quoted as the minimum number of patients required for the purposes of feasibility assessment [Lancaster et al 2004].

One of our main outcomes is safety of CRIC in patients with stroke. If we concentrate on the definition of serious adverse events (SAE) (i.e. any event that results in death, is life-threatening, requires hospitalisation or results in persistent or significant disability, or is a congenital anomaly or birth defect), then if we do not see any SAEs related to CRIC then we can be 95% confident that the true SAE rate lies between 0 and 12% [Bland M 2005].

There are no studies reporting the effect sizes of RIC on fatigue. One of the key outcomes of this pilot will be to provide information for a power calculation required for a definitive RCT of RIC to improve fatigue. However, the FSS-7 is a 7-item self-administered questionnaire that measure the effect of fatigue on various physical and social activities. Each domain is scored out of 7 with a maximum score of 49, the higher the score the greater the fatigue. The average FFS-7 score for healthy adult population is 23 points (SD0.7) [Grace et al 2006], and the reported minimal clinically important difference (MCID) in this score associated with improvement ranges between 2 and 12 for deterioration and improvement [Nordin et al 2016].

6.6. Recruitment

Potential participants will be identified from the stroke unit and stroke follow up clinics at the Royal Hallamshire Hospital, Beech Hill rehabilitation Centre, the Assessment and rehabilitation Centre at Nether Edge Hospital and by the Community Stroke Services. Any patients meeting the eligibility criteria for the study will be highlighted by clinicians working in these areas and will give these patients a copy of the study information sheet. An aphasia friendly version of the information sheet is also available. Patients will be asked whether they are happy to be contacted by a

member of the research team. Dr Ali (PI) will telephone the patient, and answer any questions they may have. If the patient meets the inclusion criteria and is happy to take part, Dr Ali will obtain verbal consent and arrange a mutually convenient time for them to attend ARC at Nether Edge Hospital, or the Clinical Research Facility or the Stroke Unit, RHH, for the first clinical meeting. This will include obtaining consent, socio-demographic and clinical data collection and baseline outcome measures.

Further information on the recruitment sources:

- Stroke unit, Royal Hallamshire Hospital – approximately 950 patients are diagnosed with stroke and are admitted to the stroke unit annually in Sheffield. There are 9 physicians who work primarily in stroke, and 3 research nurses who will be able to help identify potential participants.
- Stroke clinic, Royal Hallamshire Hospital – this reviews patients between 6 & 8 weeks following their discharge from hospital and represents a further opportunity to highlight eligible patients. All patients are screened for fatigue during their clinic visits.
- Beech Hill Rehabilitation Centre – 32 bedded unit for ongoing rehabilitation often managing patients up to 3 months after stroke.
- Community stroke service – provide rehabilitation to patients suffering stroke within their own homes after discharge from hospital. This continues for up to 3 months after stroke. Community stroke staffs are well experienced in identifying patients eligible for stroke trials.
- Assessment and Rehabilitation Centre, Nether Edge Hospital – receives approximately 140 referrals for patients with chronic stroke (after discharge from the community stroke services) annually who have ongoing rehabilitation needs.

6.7. Outcome measures

Primary outcomes – Success Criteria

1. Safety of CRIC in patients with stroke will be assessed by review of blood counts and ECGs at the end of the RIC treatment intervention, by review of the diary of side effects and investigation of any adverse events. Side effect diaries will include assessments of acceptability based on Likert scales as well as free-text sections.
    i. No serious adverse event (SAE) directly related to CRIC.
    ii. Less than 5 participants experiencing any SAE.
2. Participant acceptability will be assessed from grade (on a scale of 0-5) of discomfort associated with RIC, from review of participants' diary recordings and from the responses given by the participants undertaking the qualitative interviews with researchers.
    i. Less than 1/3 of participants reporting moderate or greater discomfort.
3. CRIC compliance will be assessed using the mobile compliance application.

i. More than 80% of intended CRIC cycles completed.

4. Study feasibility will be assessed by review of patient recruitment, outcome measure assessments recorded, and number of follow up assessments completed.
    i. Four patients recruited within the first 2 months.
    ii. >80% of outcome measure assessments and follow up assessments completed.

Secondary outcomes

The following outcome measures will be undertaken at baseline, and after the 6-week intervention period:

1. Full blood count, U&Es, LFTs, inflammatory markers (CRP, ESR)
2. Therapy time (mins).
3. Physical activity monitoring (ACTiheart, CamnTech).
4. Global physical activity questionnaire (GPAQ).
5. Six minute walk test (6MWT).
6. Peak oxygen consumption (VO2 max, ml/Kg/min).
7. Multidimensional fatigue inventory (MFI) [Donovan et al 2014].
8. Modified Rankin Score (mRS) [Bonita and Beaglehole 1988].
9. Barthel index (BI) [Mahoney and Barthel 1965].
10. PHQ9 and GAD7 [Martin et al 2006, Ruiz et al 2011].
11. MOCA [Nasreddine et al 2005].
12. EQ-5D [Van Hout et al 2012].
13. Electronic health record abstraction for vascular events and healthcare utilisation.

Secondary outcomes for a subset of patients (n=8)

The following outcome measures will be undertaken in the subset of patients who consent to having the MRI perfusion and spectroscopy studies as well as the blood analysis of biomarkers (4 CRIC and 4 sham):

1. GLP-1, heat shock protein
2. Micro-RNA analysis for pro-inflammatory and anti-inflammatory mediators and gene expression.
3. Cerebral perfusion (ml/min).
4. Cerebral metabolites (spectroscopy).

Stoller Biomarker Discovery Centre

Samples taken for biomarker analysis will be stored and sent to the SBDC in two batches (at baselines after recruitment of all participants, and after 6 week follow up visits are complete). During this time they will be stored at -20 degrees celcius in the stroke research freezer, L floor, Royal Hallamshire Hospital. They will be transported by recorded courier to Professor Anthony Whetton at the SBDC. A materials transfer

agreement for the transfer of human tissue (plasma) will be in place prior to the study start date.

Feasibility to undertake these assessments will be assumed if more than 80% of intended assessments are completed.

Qualitative Interviews – subset of 6 patients

Qualitative interviews will be undertaken to evaluate the acceptability of CRIC by asking participants to describe their expectations and experiences of the treatment. They will also investigate facilitators and barriers to use and compliance with the treatment interventions. Semi-structured interviews will be undertaken and recorded digitally and transcribed verbatim before undergoing thematic analysis [Braun and Clarke 2006].

6.8. Study Protocol

Participants that are eligible and have expressed an interest in participating in the study will be contacted by a researcher to arrange a mutually convenient time to meet with a clinical researcher. At this initial visit a consent from will be signed and then the FFS-7 completed. If the patient scores equal to or greater than 28 (overall score) they will be eligible to participate in the study. Following this, the researcher will collect baseline sociodemographic and clinical data and perform an ECG and take blood tests. Samples of blood will also be stored with patients consent for further analyses at a later date. The 6MWT and VO2 max will be undertaken in this initial visit. Participants will be randomised in a 1:1 ratio, by an online block allocation system (Sealed Envelope Ltd. 2017. Simple randomisation service) to the order in which they receive CRIC or sham intervention (approximately 17 participants to each arm). Another session within the same week will be scheduled to complete baseline assessments (questionnaires, scales, activity measures) and demonstrate use of the activity monitors and the intervention/sham procedure. The participants will then start the CRIC/sham procedure 3 times weekly for 4 weeks, and will attend the ARC, Nether Edge Hospital, or the Clinical Research Facility or the Stroke Unit, RHH, for supervision of the intervention and their routine therapy depending on their routine clinical schedule and preference. Four participants from each group will be randomly selected using the online randomisation software to undergo an MR perfusion of the brain and MR spectroscopy of the brain. This will occur at The Royal Hallamshire Hospital.

The CRIC/sham stimulus will consist of 4 cycles of 5 minutes of upper or lower (depending on tolerability) limb ischaemia followed by 5 minutes of reperfusion. This will be delivered using a manual shygnomanometer applied to the upper arm or leg and activated to go through 4 cycles (see figure 1). The blood pressure cuff in the active treatment arm will inflate to 200 mmHg (arm) and 220 mmHg (leg), while the sham arm cuff will inflate to 20mmHg. Participants will also be given a symptom diary to record general acceptability, and any untoward or unexpected effects of the

interventions. A clinical researcher will review participants at each visit to find out how things are going, review the symptoms diary, as well as the activity monitors.

Figure 1. Chronic remote ischaemic conditioning (CRIC).

After 6 weeks, a follow up face-to-face visit will be undertaken to complete follow up assessments. These will include all the assessments undertaken in the first week along with a review of hospitalisations abstracted from electronic health records. Participants will have their ECG and blood tests repeated and once again a blood sample will be stored for further analysis at a later date. The 8 participants who underwent MRI scanning will have these scans repeated. This will be the end of the study intervention. All participants will be invited to participate in semi-structured interviews during week 7 until the first six participants agree. This will further evaluate the acceptability, and feasibility of the study interventions. At 3, 6 and 12 months post randomisation a telephone follow up assessment will take place that will record mRS, EQ-5D, FFS-7, PHQ-9, GAD, and healthcare utilisation abstracted via electronic health records. Participant involvement in the study will then be complete (see figure 2).

6.9. Monitoring outcomes

The ECG, blood tests (FBC, U&E, LFT, Coagulation) will be undertaken at the initial visit and reviewed by a stroke physician. Any concerns or significant abnormalities will be referred to the principle investigator for appropriate action to ensure the safety of the participant. Weekly review of the symptom diaries by the researchers will identify adverse and serious adverse events, which will also be referred to the PI for review and action.

6.10. Safety assessments

Any adverse events will be documented in accordance with National Research Ethics Service (NRES) and investigated by the researchers for any relationship to the study intervention. These will be reviewed at a weekly research meeting with the research clinicians.

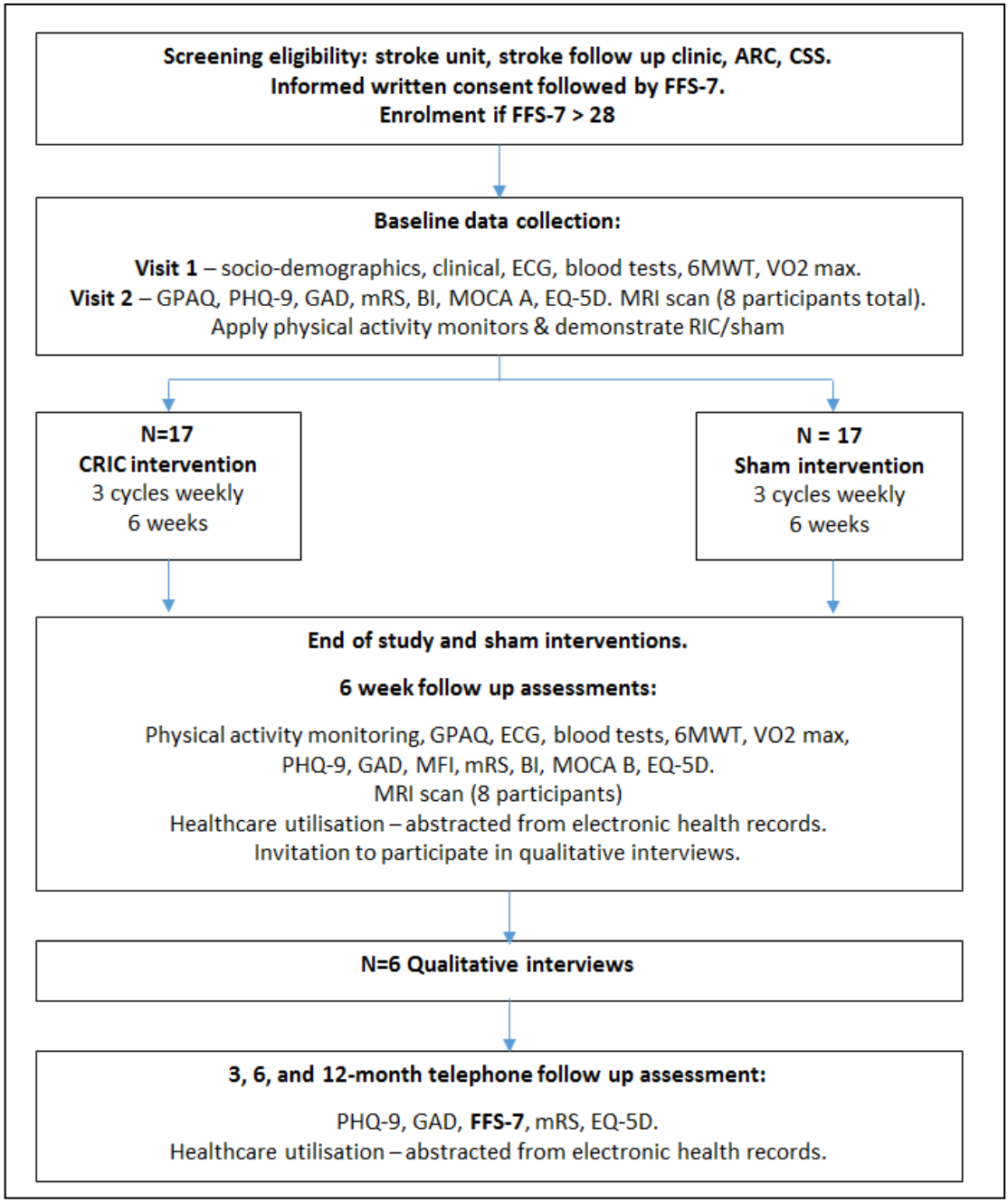
6.11. Withdrawal

Participants will be advised that they can withdraw from the study at any time without giving a reason and without this affecting their clinical care. They will be removed from the study but with their consent, data already collected will be used to help determine feasibility criteria for the study.

6.12. Data Collection Tools

All scales and measures used in this study have been validated in prior research in stroke patients. A data collection proforma will be used to record age, sex, comorbid disease, smoking and alcohol status, medication use, stroke severity (NIHSS), location, type (ischaemic or haemorrhagic), and aetiology (TOAST classification), mRS, date of onset and treatments initiated. Standardised adverse event case report forms will be completed for each participant in accordance with National Research Ethics Service (NRES) guidelines. The semi-structured qualitative interviews between consenting participants and a study researcher will be recorded using digital recording equipment and later transcribed verbatim. The researcher will use a topic guide to facilitate the interview. Copies of the assessment measures, data collection proforma and interview topic guide are attached as an addendum to this protocol.

Figure 2. Study flow diagram



6.13. Quality Control and assurance

The study will be monitored and audited in accordance with Sheffield Teaching Hospitals Standard Operating Procedures for monitoring and audit.

6.14. Project duration

Participant recruitment is anticipated to run from August 2018 to August 2020. Protocol development and submission, and ethical approval application will be completed prior to this date. It is anticipated that data analysis, thesis completion, and manuscript completion will take another 6 months (see GANTT chart).

7. Statistical opinion

As this pilot study is exploratory in nature and undertaken primarily for feasibility, only a rudimentary sample size calculation has been performed (see sample size section). The results will be mainly descriptive in nature describing safety, and acceptability of CRIC as well as feasibility of undertaking the study schedule, according to success criteria described in section 6.7 Outcome measures.

Between group differences in baseline characteristics will be compared using parametric (students t-test) and non-parametric (Mann-Whitney U, Chi square) tests depending on normality of distribution. With regards to the FFS-7 score (for fatigue), within-person differences, adjusted for appropriate baseline characteristics will be compared between CRIC and sham arms using ANOVA. Dr Karen Kilner is a medical statistician at SHU that has been consulted with regards to the study statistics.

8. Project Management

The project team (Dr Ali, Prof Majid, PhD student) will meet twice monthly to discuss problems that arise with recruitment and data analysis. The Principal Investigator will have overall responsibility for the study and will supervise, monitor and review work undertaken, the data collected and analysis performed.

Table 1. Study activity schedule.

Visit Number	Week	Assessment	Therapy	Duration (hrs)
1	1	• Screen eligibility. • Consent. • 12 lead ECG. • VO2 max. • 6MWT. • Blood tests. • MRI – 8 participants • Demographic and clinical details. • Outcome measure assessments. • Delivery and explanation of symptom diary.	• Nil	2.5 hours
2	1	• Outcome measure assessments. • Delivery and explanation of symptom diary. • Explanation of activity monitor.	• Treatment (CRIC/sham).	1.5 hours
(3-19)	1-6	• Acceptability of treatment (RIC/sham). • Symptom diary review. • Activity monitor review.	• Treatment (CRIC/sham)	1.5 hour
20	6	• 12 lead ECG. • VO2 max. • 6MWT. • Blood tests. • MRI – 8 participants. • Outcome measure assessments. • Activity monitor review. • Healthcare utilisation.	• End of study intervention.	2.5 hours
21	*7*	• *Qualitative interview*	• *Nil*	*0.75 hours*
(11) Telephone call	12	• mRS. • EQ-5D. • PHQ-9 / GAD. • FFS-7. • Healthcare utilisation	• Nil	1 hour
(12) Telephone call	26	• mRS. • EQ-5D. • PHQ-9 / GAD. • FFS-7. • Healthcare utilisation	• Nil	1 hour
(13) Telephone call	52	• mRS. • EQ-5D. • PHQ-9 / GAD. • FFS-7. • Healthcare utilisation	• Nil	1 hour

9. Expertise

Dr Ali, Dr Harkness, Professor Majid, and Dr Redgrave are consultant physicians working in the field of stroke. Dr Ali has developed the stroke exercise referral programme and has worked closely with Sheffield International Venues. Both he and Dr Harkness work with stroke patients throughout the stroke pathway and have extensive experience in recruiting to commercial and non-commercial stroke research trials as well as trials recruiting patients with dementia and those with frailty. Both also have experience supervising MSc students. Professor Majid and Dr Redgrave have supervised numerous MSc and PhD students in original research projects and have also acted as principle investigators in NIHR trials. All clinicians are involved with the University of Sheffield medical undergraduate and MSc Neurosciences course. All have expertise in undertaking and publishing research in the area of stroke. Ms Bethany Moyles is a PhD student who has conducted original stroke research under the supervision of Dr Ali for her MSc. This involved recruitment and interviewing stroke patients for their experiences of exercise after stroke . She has experience in recruiting patients to research trials, as well as coordinating a patient and public group for stroke.

The trial steering committee will include Dr Ali, Dr Nair (consultant neurologist), and Beth Moyles and a lay member (Mark Stevens). The group will meet twice yearly to ensure fidelity of the trial protocol, review recruitment and discuss any difficulties that may have arisen.

10. Ethical Issues

Safety;

The principal investigators will be responsible for investigating any adverse events in accordance with National Research Service guidelines and the study will be halted immediately by the sponsor if any concerns arise about the safety of RIC.

There have been over 39 clinical trials of ischaemic conditioning in patients with coronary artery disease and athletes [Sharma et al 2015], none of these trials have highlighted concerns over serious adverse events with regards to ischaemic conditioning.

Confidentiality;

Participant information (socio-demographic, clinical, outcome measure assessment) will be recorded on paper clinical research forms that will be furnished with a study number rather than the patient identifiers. This will then be transferred to an encrypted electronic database stored on a password protected Sheffield Teaching Hospitals research laptop, access to which will be restricted to researchers entering data and performing statistical analyses. Each study participant will be assigned a unique ID number, which will be the only "identifier" linked to data collected. A separate enrolment log will be kept updated by the researcher and held within a study file separate to the study site file. This will link these numbers to the participant's

personally identifiable information. In this way, the participants' data will be "pseudo-anonymised" and no identifiable information will be kept with the actual study data.

The research laptop, study site file and enrolment log will be stored in a locked office at Sheffield Teaching Hospitals, RHH. Participants will undergo baseline and follow up data collection at the ARC, Nether Edge Hospital, CRF or SU at the RHH. Rooms there are private and data collection will be conducted in a confidential manner.

Six patients will also undergo semi-structured interviews. Interview guides and paper notes will also be kept in the study site files in the pseudo-anonymised manner. Audio recordings of the interviews will be stored digitally on an SD memory card, which will be broken to destroy data once the interviews are transcribed. Interviews will be undertaken in clinic rooms at STH that are also private. All interviews will be conducted in a confidential manner. The transcribed recordings will be anonymised and labelled using the study ID. The files containing the transcriptions of the interviews will be stored electronically but accessible only to members of the research team on password protected computers. When required, the files will be shared between research team members using a password protected flash drive, and copies will be deleted from the flash drive when no longer required. A printed copy of each transcript will be stored in the Study Site File after the study and the Site File will be kept securely on hospital property.

The qualitative report is likely to contain direct quotations from the study participants but these will be anonymous and the participant will not be identifiable from the quotations used.

Consent; Patients will only be enrolled in the study if they have capacity to understand the study information sheet and give informed consent. All members of the research team have undergone GCP training, Participants are free to withdraw from the study at any time without giving a reason and without it affecting their clinical care.

Participants will be free to undergo all other therapeutic activities (e.g. stroke rehabilitation, exercise, psychological therapies) during the study period and will be asked to record this in their study diaries.

Travel Reimbursement; Participants will be reimbursed expenses of travel to and from scheduled study visits.

11. Service Users

A patient and public panel was consulted on the 6th September 2016 about practical aspects of undertaking the study (e.g. how to approach eligible patients, how long after stroke to include patients, the feasibility of undertaking the required outcome measure assessments) as well as reviewing the associated documentation (e.g. consent forms, participant information sheets, invitation letters etc). The panel, that included 8 members who had suffered stroke or were the main carers for those with stroke, commented positively on the need for such a project and provided numerous

suggestions that ultimately resulted in changes to enhance the success of the study. These included:

- Reducing the number of outcome measures undertaken in one sitting. Following this suggestion, baseline data collection and assessments were divided into 2 sittings. Further, carers / relatives will be able to assist participant completion of assessments if significant language impairment existed.
- Production of an aphasia friendly participation information leaflet.
- Increasing the number of eligible participants to include patients at any time after stroke. This was in response to many of the participants reporting ongoing feelings of fatigue after 12 months post stroke.
- Ensuring baseline and follow up assessments are undertaken according to when patients are maximally alert, depending on participant preference. Some patients are more fatigued in the afternoons / early evenings, however some will be more fatigued in the mornings and as such, the option of afternoon assessments will be offered.

In addition one of the PPI lay members has agreed to become a member of the trial steering committee (Mark Stevens). They will join Dr Ali, Dr Nair and Beth Moyles (PhD student) twice yearly to discuss study progress.

12. Dissemination

The study will aim to recruit a PhD student (University of Sheffield) to facilitate all aspects of the research project from screening through to recruitment, intervention delivery, data analysis and thesis write up. The study may take up an MSc postgraduate student (e.g. Neurosciences MSc, University of Sheffield) to help with data collection or analysis, or a particular aspect of the study during its course. In both instances the student/s will submit their dissertations and present their findings to the Neurology and Stroke department. The overall findings of the study will also be presented locally to the academic directorates of Neurosciences and the Combined Community and Acute care group. The research team will also aim to present the findings at international scientific meetings and will write up the findings for publication in peer-reviewed journals such as Stroke, Clinical Rehabilitation and Archives of physical medicine and rehabilitation. The study will also be registered on the Sheffield Teaching Hospital NHS Foundation Trust research database, and be advertised on the University of Sheffield website under the Biomedical Research page.

13. Taking the Work Forward

With the pilot data generated from this project and the outcome from working with patients and public in the development of the proposal, we plan to submit an application to fund a trial involving a larger number of participants to evaluate the effect of CRIC on fatigue as well as improving physically activity after stroke, with sample size calculations for power to detect clinically meaningful improvements in outcomes such as fatigue, and peak oxygen consumption (VO2). We intend to apply to the NIHR to fund this trial.

We will also look at any effect of CRIC on vascular risk (major cardio and cerebrovascular events abstracted from electronic health records) at 3, 6 and 12 month follow up to determine whether any signal indicating a secondary protective effect can be seen. If this is the case then a separate application for funding to evaluate CRIC in secondary vascular risk prevention will be developed.

14. Intellectual Property

RIC protocols have been used in many studies previously, and the current protocol is based on those most commonly used. CRIC has been used in a Chinese stroke population [Meng et al 2012]. Discussion with Medipex regarding any IP developed with the stroke population are ongoing.

15. Costing

- Travel expenses
- Research assistant time
- Room hire ARC
- Recording equipment
- Physical activity monitors
- Blood tests
- ECG analysis
- Stationary / consumables
- Statistician time
- Equipment for VO2 max measurement
- Qualitative interviewing
- Online randomisation
- Lab set up fee
- Research and development administration
- Results dissemination

16. Funding arrangements

We have been successful in an application for seed funding from the Ryder Briggs Trust to help fund this trial (£34,526.24). We have also received a further £9,500 from the stroke research fund. This has been met with funding from the Biomedical

Research Centre funding (£40,000) to put towards the PhD stipend and fees. We will aim to incorporate 1-2 Neuroscience MSc students over the course of the study that will help with extra lab fees.

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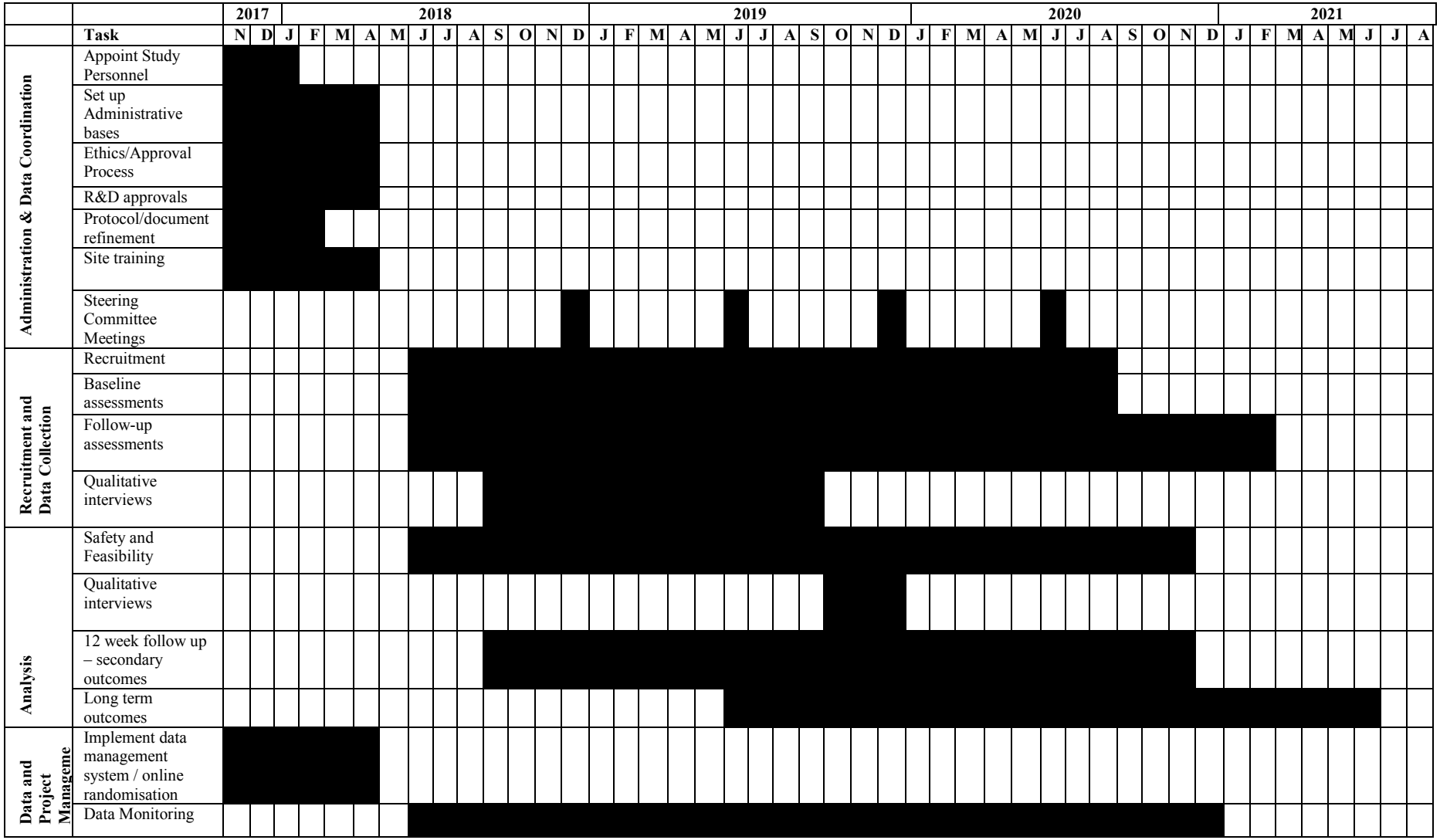
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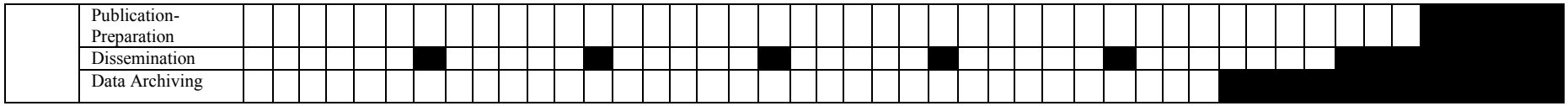
Set up Administrative bases
Ethics/Approval Process
R&D approvals
Protocol/document refinement
Site training
Steering Committee Meetings
Recruitment and Data Collection
Recruitment
Baseline assessments
Follow-up assessments
Qualitative interviews
Analysis
Safety and Feasibility
Qualitative interviews
12 week follow up – secondary outcomes
Long term outcomes
Data and Project Manageme
Implement data management system / online randomisation
Data Monitoring



GANTT Chart.