Biomarkers of stroke recovery: Consensus-based core recommendations from the Stroke Recovery and Rehabilitation Roundtable (SRRR).

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Keywords (3-7 total): Biomarker, motor, somatosensory, cognition, language, stratification, function, clinical trial, neuroimaging, human

Cover Title: Biomarkers for Stroke Recovery

Word count: 4156 without references

List of tables and figures:

Abstract

The most difficult clinical questions in stroke rehabilitation are "What is this patient's potential for recovery?" and "What is the best rehabilitation strategy for this person, given her/his clinical profile?" Without answers to these questions, clinicians struggle to make decisions regarding the content and focus of therapy, and researchers design studies that inadvertently mix participants who have a high likelihood of responding with those who do not. Developing and implementing biomarkers that distinguish patient subgroups will help address these issues and unravel the factors important to the recovery process. The goal of the present paper is to provide a consensus statement regarding the current state of the evidence for stroke recovery biomarkers. Biomarkers of motor, somatosensory, cognitive and language domains across the recovery timeline post-stroke are considered, with the exclusion of blood and genetics. We provide evidence for biomarkers that are considered ready to be included in clinical trials, as well as others that are promising but not ready and so represent a developmental priority. We conclude with an example that illustrates the utility of biomarkers in recovery and rehabilitation research, which demonstrates how the inclusion of biomarkers will enhance future clinical trials. In this way, we propose a way forward for when and where we can include biomarkers to advance the efficacy of the practice of, and research into, rehabilitation and recovery after stroke.

Introduction

Stroke is a heterogeneous condition, making choice of treatment, and prediction of outcome and treatment response, difficult. Despite this, clinical trials are often designed with a 'one size fits all' point of view, which can make them vulnerable to patient heterogeneity, reduced statistical power, and thus failure. Biomarkers can greatly inform patient selection for trials in general medical research, and this is equally true for stroke recovery. A stroke recovery biomarker (SRB) can be defined as an indicator of disease state that can be used as a measure of underlying molecular/cellular processes that may be difficult to measure directly in humans, and could be used to understand outcome, or predict recovery or treatment response (1).

In practical terms biomarkers should improve our ability to predict long-term outcomes after stroke across multiple domains. This is beneficial for: a) patients, caregivers and clinicians; b) planning subsequent clinical pathways and goal setting; and c) identifying whom and when to target, and in some instances at which dose, with interventions for promoting stroke recovery (2). This last point is particularly important as methods for accurate prediction of long-term outcome would allow clinical trials of restorative and rehabilitation interventions to be stratified based on the potential for neurobiological recovery in a way that is currently not possible when trials are performed in the absence of valid biomarkers. Unpredicable outcomes after stroke, particularly in those who present with the most severe impairment (3) mean that clinical trials of rehabilitation interventions need hundreds of patients to be appropriately powered. Use of biomarkers would allow incorporation of accurate information about the underlying impairment, and thus the size of these intervention trials could be considerably reduced (4), with obvious benefits. These principles are no different in the context of stroke recovery as compared to general medical research (5).

Interventions fall into two broad mechanistic categories: 1) behavioural interventions that *take advantage of* experience and learning-dependent plasticity (e.g. motor, speech and language therapy), and 2) treatments that *enhance the potential for* experience and learning-dependent plasticity to maximise the effects of behavioural interventions (e.g. pharmacotherapy or non-invasive brain stimulation) (6). To identify in whom and when to intervene, we need biomarkers that reflect the underlying biological mechanisms being targeted therapeutically.

Our goal is to provide a consensus statement regarding the evidence for stroke recovery biomarkers that are helpful in outcome prediction and therefore identifying subgroups for stratification to be used in trials (7). We focused on stroke recovery biomarkers that can investigate the structure or function of the brain (Table 1). Four **functional domains** (motor, somatosensation, cognition, and language [Table 2]) were considered according to **recovery phase** post stroke (hyperacute: <24hrs; acute: 1 to 7 days; early subacute: 1 week to 3 months; late subacute: 3 months to 6 months; chronic: > 6 months (8)). For each functional domain, we provide recommendations for biomarkers that either are: 1) ready to guide stratification of subgroups of patients for clinical trials and/or to predict outcome, or 2) a developmental priority (Table 3). Finally, we provide an example of how inclusion of a clinical trial-ready biomarker might have benefitted a recent phase III trial. As there is generally limited evidence at this time for blood or genetic biomarkers, we do not discuss these, but recommend they are a developmental priority (9-12). We also recognize that many other functional domains exist, but focus here on the four that have the most developed science.

A challenge across the reviewed literature was to determine where biomarker data explained recovery beyond that denoted by clinical outcome measures, such as the proportional recovery rule that has been demonstrated using motor (3, 13), visuospatial

neglect (14) and language (15) outcomes. Given the recency of these models, we were unable to address this in this paper and urge people to consider this in their future trial design. Further, to fully understand the predictive capacity of biomarkers we need to move beyond cross-sectional studies, which constitute the bulk of the biomarker literature at present, and conduct mechanistic studies that go beyond simple correlations as well as longitudinal studies that provide data useful for predicting outcome or treatment response.

Motor

Neuroimaging biomarkers include quantitative characterisation of the stroke lesion itself, as well as the structure and function of non-lesioned brain areas. There is little consensus regarding the usefulness of characterizing the ischemic penumbra at the **hyper-acute** stage in relation to predicting motor outcomes. Recent data suggest that the site of ischemic penumbra, rather than volume, could predict outcome or treatment response (i.e. to thrombolysis) and affect motor recovery (16). **Acute** infarct volume correlates with motor outcome (National Institute of Health Stroke Severity [NIHSS]), but this relationship is attenuated with increasing leukoaraiosis severity (17, 18). The extent of existing white matter disease (i.e. leukoaraiosis) has been associated with **acute** lesion size, degree of lesion expansion and stroke severity indicated by initial NIHSS score (19). These findings underscore the point that biomarker performance varies across different stroke subgroups.

Measures of corticospinal tract (CST) white matter integrity in the **acute** stage may predict motor outcome. Early measurement of CST fiber number via diffusion tensor imaging (DTI), a reflection of white matter integrity, predicts motor outcome (Fugl-Meyer score) at 12 months, especially for patients with initially more severe impairment (20). Other data (21), though not all (22), also suggest that fractional anisotropy (FA) of the ipsilesional and contralesional CST at the **acute** stage is higher in individuals who achieve better motor

recovery after stroke. Data also support the potential utility of the asymmetry between the ipsilesion and contralesional CST FA to predict treatment gains in the **chronic** stage (23).

Measures of the extent of CST injury in the **acute** stage, such as via corticospinal tract lesion load (24), also have predictive value for poor motor outcome. A model including this biomarker improved prediction of Fugl-Meyer motor score at 3 months post-stroke that was beyond what could be determined by baseline behavioral assessment, age or infarct volume. Several studies have found that in the **chronic** stage the extent of CST injury also helps predict treatment gains (25-27).

Other regions distant from the lesion influence motor recovery. While measures related to distant regions require further development to reach the stage of valid biomarker, several useful observations have been published in the **chronic** stage, including those related to contralesional CST (28), corpus callosum (29), precentral gyral (30), and superior longitudinal fasciculi (31, 32). Multivariate machine learning methods have recently been applied to neuroimaging data with the aim of providing individual predictions based on an approach that integrates features extracted from brain voxels from multiple brain areas, rather than one area (33). In patients presenting with severe upper limb impairment, classification of a subsequent good or poor recovery was more accurate using lesion information from a range of cortical and subcortical motor-related regions compared to just using CST (87% compared to 73% accuracy respectively) (34). Such approaches emphasize the importance of taking account of damage in multiple brain regions, extending beyond CST, in order to best understand variation in motor outcome (34-36).

There is broad consensus that the presence of an upper limb motor evoked potential (MEP) in response to transcranial magnetic stimulation (TMS) at the **hyperacute** and **acute** stages strongly predicts good motor outcome (37, 38) and that shorter motor evoked potential (MEP) latencies and central motor conduction times are associated with better outcome (39).

The presence of a MEP has been found to identify which patients will follow the proportional recovery rule (40). Similarly, in the leg, the presence of a MEP indicates that an individual is more likely to be independently mobile 12 months post-stroke (41-43), yet this measure does not relate to walking recovery (13). Prediction of recovery is more challenging for patients without a MEP (36, 40) and combining TMS with MRI biomarkers may be useful in this context (38) TMS at the **chronic** stage helps explain the relationship between corticomotor function and motor performance in cross-sectional studies, and those who have a MEP are more likely to benefit from physical interventions (23, 44, 45).

Resting state functional connectivity (rsFC) findings in the **early** and **late subacute** phases converge on the conclusion that interhemispheric connectivity is of particular importance to motor control. Cross-sectional studies have demonstrated decreased rsFC correlates with the degree of motor impairment (46, 47). The are positive associations between **acute/early subacute** rsFC (ipsilesional primary motor cortex [M1] to contralesional thalamus, supplementary motor area [SMA], and medial frontal gyrus) and motor outcomes at 6-months (Fugl-Meyer score) (48). In **late subacute** patients, the amount of CST damage combined with interhemispheric M1 rsFC best predicted therapy-induced gains (27). Fan et al. (49), found that in **late subacute** patients change in interhemispheric M1-M1 rsFC predicts improvements in the Wolf Motor Function Test. Results from a ridge regression machine-learning algorithm analysis of a large sample of **early subacute** stroke patients suggest that rsFC may explain a smaller amount of the behavioral variance observed than the amount of structural damage to the CST (50). As rsFC can be performed in patients with severe deficits after stroke and can interrogate all brain networks simultaneously, it represents a priority for development.

Quantitative indices extracted from functional MRI (fMRI) in the **early** and **late subacute** stage, such as the laterality index from M1, and the study of its change over time,

show that stroke is associated with a less lateralized pattern of activation as compared to healthy subjects, a finding that is exaggerated among patients with poorer outcomes (51, 52). One unifying conclusion across studies is that the best motor outcomes are associated with the greatest shift towards the normal state of brain function (53). The laterality index has been used as a judgment criterion of efficacy in the **chronic** stage in trials testing mirror therapy (54), constraint-induced therapy (55) and robotic intervention protocols (56), and can predict treatment response (57). Other fMRI metrics such as activation volume (58, 59) or percent signal change (60) within key motor network nodes can predict response to treatment in the **chronic** stage. As there are fewer long-term studies of the laterality index, and it often shows significant biological associations, this is an area of priority for development.

Magnetoencephalography (MEG) or electroencephalography (EEG), non-invasive measures of cortical neuronal oscillations, are sensitive to alterations in both GABAergic and glutamatergic signalling that are important for plasticity and recovery after stroke (61-63). Changes in cortical excitation and inhibition represent novel therapeutic targets, but cannot be measured directly in humans. Stroke patients with poorer outcomes have persistent, increased low-frequency oscillations at the **acute**, and **early**/**late subacute** stages (64); suggesting predominant inhibitory mechanisms in the peri-lesional cortex. **Acutely**, lower beta-rebound in response to tactile finger stimulation (which indicates increased early post-stroke sensorimotor excitability) (65) and increased somatosensory map size (66) predict good recovery after stroke. Also, in a single stroke patient, zolpidem *reversed* increased peri-lesional theta (4-10Hz) and beta oscillations leading to clinical improvement (67). In the **chronic** phase, dense array EEG was able to predict motor gains from a 4-week intensive training program (68). While MEG/EEG cannot currently be recommended to guide subgroup stratification in trials at present, this is a developmental priority.

Combining neuroimaging and neurophysiology biomarkers may be useful for predicting motor outcomes and therapy response (69). Upper limb outcomes at 3-months can be predicted at the **early sub-acute** stage by measuring first clinical, then TMS and finally MRI biomarkers in a stepdown approach, as in the PREP algorithm (70, 71). Stoykov & Stinear (72) treated **chronic** stroke patients using active-passive bilateral arm training and discovered that fractional anisotropy asymmetry between the two CST tracts accounted for 40% variability in clinical improvement. Factoring in whether patients were MEP+/- improved the predictive model. One recent study emphasized that combining neuroimaging measures of neural injury and neural function was key to best predicting response to a standardized robotic therapy in the **chronic** stage (27).

In summary, neuroimaging and neurophysiology CST biomarkers can predict motor outcome and response to therapy after stroke, and are recommended for use in clinical trials, e.g., for stratifying patients. The evidence for rsFC, fMRI and MEG/EEG biomarkers are promising and are developmental priority areas (Table 4A).

Somatosensory

Currently there are few studies of structural or functional biomarkers conducted to understand outcome, predict recovery or predict treatment response in the somatosensory functioning domain in the **hyperacute or acute** phases post-stroke. Most work on structural biomarkers involving the non-lesioned brain has focused on understanding outcome by mapping the structural integrity of residual pathways. Feasibility of visualisation of sensorimotor systems by tracking fibres has been demonstrated in **hyperacute**, **acute** and **early subacute phases** for somatosensory symptoms (73). There are changes in morphology of the somatosensory cortex of **chronic** stroke patients (74), with co-localized structural (cortical thickness) and functional (brain activation, tactile stimulation) effects. Only one

study noted associations between the structure of somatosensory regions and motor outcome in the **chronic** phase (75).

Failure to activate the somatosensory cortex during median nerve stimulation in the **acute** stage predicts poor clinical recovery at 3 months (76). Using MEG, reduction in interhemispheric asymmetries of activity at **chronic** compared to **acute** phases was associated with a worse clinical state (77). Studies using MEG in **early** and **late subacute** phases show that changes in source strength of the primary somatosensory cortices correlate with the extent of recovery of sensorimotor functions as determined by neurological exams (e.g., graphesthesia test) (78). Yet, MEG can be complicated to employ, and so broader evaluation of these findings might benefit from use of less complex electrophysiologial methods such as EEG. In the **subacute** phase differences in brain activity measured with task-related fMRI correlated with touch impairment in patients with thalamus /internal capsule lesions compared to those with lesions of primary (SI) or secondary (SII) somatosensory cortex (79). Similarly, responsiveness of SI at 1-15 days post-stroke is associated with improvement of two-point discrimination 3 months post-stroke (80). Resting-state FC studies of touch impairment and recovery demonstrated a correlation between changes in connectivity from contralesional SII and contralesional inferior parietal and middle temporal gyrus with changes in a tactile discrimination score that were absent in a control group (81).

Associations are observed between somatosensory function (using the Hand Active Sensation Test) and a left/right FA ratio from the sensory component of the superior thalamic radiation in the **chronic** stage (82) and the frontoparietal tracts in the **acute** (83) and **chronic** (84) phases. In addition, somatosensation function in the **chronic** phase correlates with activity in the ipsilesional and contralesional primary sensorimotor cortex (85) and a more distributed pattern of activity involving parietal cortex (82). Improvement in touch

discrimination at 6 months was associated with increased rsFC between seeds in the contralesional hemisphere and distributed regions, including cerebellum (81). Using MEG, involvement of ipsilesional primary hand representation areas positively contributed to clinical recovery (86).

Changes have also been reported in association with training of touch discrimination (87), passive proprioception (88) and sensorimotor function (89), with a focus on tracking outcomes and mechanisms, rather than prediction. For example, touch discrimination training of patients with somatosensory loss in the **chronic** stage post-stroke was associated with different patterns of change in activation with thalamic/capsular compared to SI/SII cortical lesions (90). This area of research is a priority for development.

There is insufficient evidence to recommend the use of any specific biomarkers of somatosensory system function in clinical trials. This is in part related to the complexity and diversity of somatosensory signals and their measurement. The recovery of somatosensation is often overlooked despite well-documented observations that impaired sensation is an impediment to optimal recovery (91-93). Functional biomarkers, including task-related activation and rsFC are a developmental priority (Table 4B).

Cognition

Mapping executive/cognitive functions to specific brain regions is problematic because these functions are distributed widely across broad brain networks, and their relationships are complex. Indeed, studies that readily identify structure-function relationships for phonology and semantic processing, and often fail to find an equivalent for executive function (94). The most consistent relationships were found in white matter. Frontal and basal ganglia region microbleeds were associated with executive dysfunction outcome in the **chronic** phase (95), and another study found that mean diffusivity of normal

appearing white matter (whole brain) in non-lesioned areas correlated with outcomes for executive function among individuals with ischaemic leukoaraiosis (plus a previous lacunar stroke) (96, 97). EEG changes in frontal lobes paralleled behavioral gains across multiple cognitive domains in one study that used intensive video gaming in health adults (98); the same may extend to patients with stroke.

Though functional imaging methods may offer the best hope of generating robust biomarkers for executive function, there is little published work. Available associations are correlative and from cross-sectional studies, rather than predictions of outcomes or more complex evaluation of biological hypotheses. In the **late subacute** phase, executive functioning correlates with alpha band functional connectivity between the left fronto-opercular cortex and the rest of the brain (99). Yet, it is possible that the task-dependent changes observed with functional imaging data have less to do with new domain-specific areas being generated, and more to do with cognitive control networks improving residual performance (100). In individuals with **chronic** post-stroke aphasia a positive correlation between task-dependent activity in midline frontal cortex and language recovery was interpreted as reflecting domain-general cognitive control systems (101), a finding that is consistent with training effects in healthy elderly subjects (98). Development of biomarkers in this context would likely foster advances in therapeutic techniques to train executive function, a key priority that is feasible (102).

The default mode network (DMN) has emerged as a key bioligial substrate in the context of cognitive functioning (103). Studies in the **subacute** and early **chronic** phases report altered rsFC in the DMN correlated with cognitive performance after stroke (104-107) Re-emergence of the anticorrelation between the DMN and task-positive networks, such as the dorsal attention network (DAN) (108), is associated with behavioural recovery of cognitive functions. Resting state studies have provided robust examples of disruption of

interhemispheric connectivity associated with domain-specific cognitive deficits (46, 50, 109, 110) and recovery (111). In a small longitudinal study of stroke patients compared to healthy controls, He at al (2007) showed a robust correlation between left-right posterior intraparietal sulcus rsFC and accuracy of detection of targets presented in the left neglected visual field. Multiple studies have confirmed this connectivity is much depressed in the acute stage after stroke in persons with USN and returns toward normal in association with the resolution of symptoms, with the largest current longitudinal study retaining 64 stroke patients at 12 months (111). Similar relationships have been reported for the DMN and other networks (46, 50, 109, 110). While correlational analyses cannot establish causality and do not provide the predictive functions required of an effective stroke recovery biomarker, the finding that a change in rsFC correlates with behaviour lends support to the idea that measures of network connectivity have the potential to serve as useful biomarkers across multiple behavioural domains, a possibility that requires further studies.

In the domain of spatial cognition, multiple moderately sized studies of right hemisphere injury confirm that damage to different major long range white matter tracts may predict chronic persistence of unilateral spatial neglect. Two well designed longitudinal studies implicate the inferior occipitofrontal fasciculus and uncinate fasciculus (112) and decreased FA in the left and right superior longitudinal fasciculus II, and forceps major of the corpus callosum with neglect scores. In cross-sectional studies, linear regression shows an association of unilateral spatial neglect with damage to the fronto-parietal segment of the arcuate fasciculus, and that 78.9% and 81.6% of patients with neglect had damage to the superior longitudinal fasciculus II and superior longitudinal fasciculus III respectively compared to only 15% and 30% in patients without neglect (113). While damage to superior longitudinal fasciculus III, arcuate fasciculus, frontal aslant, and frontal inferior longitudinal fasciculus are increasingly implicated in abnormal spatial cognition (114). Whether damage

to any of these white matter structures as measured in the acute phase of stroke can serve as a biomarker for persistence of USN in the chronic phase, or in stratifying or selecting patients for interventions is yet to be determined.

Based on this evidence, biomarkers of cognitive function, including executive functioning, are not ready for immediate broad implementation in clinical trials. Thus, further study and validation of biomarkers that explain current state and future chante in cognitive functions are a significant priority area for development. Resting state FC is a promising candidate biomarker (Table 4C), and study of its utility as a biomarker of recovery is emphasized here.

Language

There are a number of studies identifying a relationship between lesion site and aphasia (115), anatomical findings that suggest potential metrics to evaluate as biomarkers. In the **hyperacute period**, perfusion-weighted MRI showed that word comprehension deficits are strongly correlated with blood flow within Wernicke's area (116). A related study demonstrated that lexical processing was more strongly related to the volume of hypo-perfused tissue than the volume of lesion (117). Imaging illustrates that recovery of word comprehension from the hyperacute to acute phase (3 days) is associated with reperfusion of Wernicke's area (118). Recovery of naming in the **hyperacute** period is predicted by reperfusion of left posterior middle temporal/fusiform gyrus, Broca's area, and/or Wernicke's area (119-121). There are no established predictors of long-term (>3-days) recovery from biomarkers assessed in the **hyperacute** period (<24 hours); thus, this is a developmental priority.

Impaired repetition in the **acute** phase was associated with structural damage to the arcuate fasciculus and Broca's area as well as tissue dysfunction (hypoperfusion and frank

damage) in the inferior portion of the left supramarginal gyrus and temporal-parietal junction (122). Kummerer et al., (2013) also observed that impaired repetition at this phase was associated with posterior temporal-parietal lesions and damage to the dorsal superior longitudinal and arcuate fasciculus, while comprehension deficits were associated with ventral extreme capsule fibre damage (123).

In the early **subacute phase**, there are relationships between lesion location and aphasia symptoms that suggest potential biomarkers. Kriesler et al. (2000) correctly classified 67% to 94% of patients based on analysis of lesion location and symptoms (124). Forkel et al., (2014) demonstrated prediction of recovery at 6 months was improved by adding volume of the left long segment of the arcuate fasciculus to a regression model including age, sex, and lesion size (125); including volume of the right long segment of the arcuate fasciculus further improved recovery prediction. Recent work by Geranmayeh et al., (126) showed that propositional language production is predicted by interactions between brain networks (DMN, fronto-temporo-parietal, and cingulo-opercular networks) rather than by activity within a single individual network highlighting the distributed nature of language operations. Functional MRI activity in the early **subacute phase** shows promise as a predictor of long-term recovery when analyzed using a multivariate machine learning technique. Saur et al., (2010) employed this method with a mask of task-induced fMRI activity in bilateral frontal and temporal regions in combination with behavioural language performance and age (127). This approach correctly predicted good versus poor language recovery in 86% of individuals with stroke who had aphasia at 2 weeks. In the largest case-control study of subacute stroke patients with aphasia to date, Yang et al (128) found that in patients with lacunar stroke, interhemispheric rsFC was increased in the superior temporal gyrus, the inferior frontal gyrus and the lingual gyrus. Of note is the observation that the interhemispheric hyperconnectivity of the superior temporal gyrus was inversely correlated with the aphasia quotient, indicating

that greater connectivity was associated with worse aphasia.

Voxel-based analyses in the **chronic** phase has established utility in multiple brain systems and specifically have identified structural damage associated with particular aphasic symptoms, distinguishing between semantic and phonological processes and recognition versus production (129-131). Arcuate fasciculus lesion load negatively influences speech production (132) and classifies severe and non-severe outcomes with 90% accuracy for naming and 96% accuracy for speech fluency (133). The PLORAS (Predicting Language Outcome and Recovery After Stroke) system (134) uses a Gaussian process model regression with a large database of stroke patients (from 1 month post, therefore covering early and late subacute, and chronic phases) with structural MRI, demographic, and language performance to provide predictions of aphasia recovery at the individual level. Using this approach and covariate factors of time of stroke, volume, and 35 different brain regions, predictions of language outcome, and within subject changes in speech production, have been identified (135). This method has high potential to provide measures that can serve as biomarkers to predict recovery.

Posterior middle temporal lobe damage can negatively affect aphasia therapy outcome in the **chronic** phase (122). Meinzer et al., (2010) observed a negative relationship between the proximity of the lesion to the hippocampus and response to a naming treatment (136). Bonilha et al., (2016) showed that measures of neural network connectivity combined with initial behavioral deficit severity accounted for 78% of variance in response to anomia treatment (137). Several small studies identified a relationship between therapy success and integrity of the left arcuate fasciculus (138), right arcuate fasciculus (139) and white matter in proximity to the hippocampus (136). Further, several fMRI studies have investigated treatment-induced aphasia recovery, predominantly in the **chronic** stage. Fridriksson (2010) identified a significant relationship between treatment-induced naming improvements and

fMRI activity in a both a posterior cluster (including parietal lobe and precuneus) and an anterior cluster (including middle frontal gyrus and pars opercularis) (122). Subsequent analyses (140) showed that altered activity in perilesional areas was associated with increased naming accuracy, but measures of pre-treatment brain activity (as opposed to changes in activity) predicted improvement in semantic errors, suggesting additional factors contribute to treatment outcome.

In summary, in the **acute and early subacute** stages, the use of structural MRI and DTI provide insights into the neural basis of language deficits, but there are not sufficient large studies demonstrating that these methods clearly improve prediction of recovery or treatment response. Functonal brain assessments such as via fMRI show potential at the **early subacute** stage for significantly improving prediction of outcome (127), however, this approach needs validation. Meanwhile, resting state interhemispheric connectivity may be increased in those most severely affected. This change in connectivity is in the opposite direction of what has been reported for other distributed networks after stroke, a discrepancy which must be reconciled. Structural MRI and DTI may forecast recovery at the **late subacute and chronic** stage, suggesting the possible use of these techniques to stratify patients for clinical trials, understand therapy mechanism and predict outcome. It should be noted that: (1) there is still considerable variability in outcome that is not accounted for by these methods, (2) each method uses a unique and complex analysis technique, (3) different aphasia treatments may engage unique networks (Table 4D), and (4) detailed studies examining the combined utility of anatomical and functional brain measures for predicting language recovery are warranted.

Conclusions

How might biomarker data be incorporated into future stroke recovery research? As a first point, the term "stroke" is inadequate, as it describes a very heterogeneous group of disorders that are unified by a vascular injury, but not by size, location, or impact of injury. Biomarkers present a way forward to subgroup or stratify patients in order to reduce variance and increase power, allowing for smaller sample sizes (7). Moreover, the final behavioral phenotype after stroke can arise from many different biological states, which could result in differential therapeutic responses; functional measures are complementary to anatomical/injury measures. Thus, a patient exploiting all possible compensatory brain mechanisms might have little room to improve, while a similar patient who uses no compensatory mechanisms might achieve benefit (141). Furthermore, inclusion of appropriate biomarkers may improve the ability to disentangle treatment responders from non-responders.

Clinical trials therefore need to base participant eligibility on more than presence of a stroke, or behavioural status. Instead, patient selection should include appropriate biomarkers; ideally these will be linked with preclinical methods as well as the biological mechanism of the therapy or treatment under investigation. For example, recently a threshold was defined whereby no patient in the **early** and **late subacute** stage with >63% injury to the CST achieved clinically important gains associated with a robotic therapy (27). This result highlights the ascendant role that neuroimaging measures need to play in clinical-decision making for post-stroke rehabilitation (142).

A useful example comes from the recent phase III Everest trial (143), which relied on behavioural assessments to determine participant eligibility, and ultimately found that patients randomized to epidural motor cortex stimulation did not reach the primary efficacy endpoint more often than patients in the control group. However, a *post hoc* analysis of patients randomized to epidural stimulation found that the primary efficacy endpoint was

reached more often (67%) by those with preserved motor evoked responses upon cortical stimulation compared to those lacking a response (27%) (26). Thus, had confirmation of physiological integrity of the biological target been an eligibility criterion (as was the case in all preclinical studies that were translated to generate this trial), the effect size would have been substantially higher and the trial results quite different. We believe that this example is highly useful in illustrating the utility of biomarkers in recovery and rehabilitation research and expect that the inclusion of biomarkers will enhance future clinical trials.

Acknowledgements: We would like to acknowledge Prof Julie Bernhardt for convening this first Stroke Recovery and Rehabilitation Roundtable (SRRR), Ms Katie Lafferty for moderating discussions at SRRR and Dr Karen Borschmann for project management. We thank Prof Leonid Churilov for his assistance with scoping survey design and analysis. The Florey Institute of Neuroscience and Mental Health was the coordinating centre, for which we acknowledge support from the Victorian Government, in particular from the Operational Infrastructure Support Grant. Financial support was provided by NHMRC CRE Stroke Rehabilitation & Brain Recovery (Australia), Heart and Stroke Foundation of Canada, and Heart and Stroke Foundation Canadian Partnership for Stroke Recovery. An unrestricted educational grant was provided by Ipsen Pharma.

LAB was supported by the Canada Research Chairs and MSFHR (CI-SCH-01796); KSH was supported by NHMRC (1088449) and Micheal Smith Foundation for Health Research (MSFHR) (15980); SCC was supported by a grant from NIH (K24 HD074722).

SCC was supported by K24-HD074722.

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Table 1: Summary of possible brain biomarkers to measure structure or function.

Measures of structure/injury:	Measures of function:
• Computed tomography (CT) • Diffusion tensor imaging (DTI) • Fluid Attenuation Inversion Recovery (FLAIR) • Gradient echo and spin echo (GRASE) • T1-weighted MRI • T2-weighted MRI • Proton density-weighted MRI	• Electroencephalograpy (EEG) • Functional magnetic resonance imaging (fMRI) • Magnetoencephalography (MEG) • Magnetic resonance spectroscopy (MRS) • Near Infrared Spectroscopy Imaging (NIRSI) • Positron emission tomography (PET) • Resting state magnetic resonance imaging (rsMRI) • Sensory electroencephalography (sEEG) • Transcranial magnetic stimulation (TMS)

Table 2: Scope of functional domains considered given existing literature.

Motor refers to motor outcomes, inclusive of upper and lower limb functions.

Sensation refers to somatosensation, touch and proprioception.

Cognition refers to 'executive functioning' or 'cognitive control', which are umbrella terms for sub-processes of selective attention, error monitoring, decision-making memory and response inhibition.

Language refers to spoken language production, auditory language comprehension, and global measures of language function (that also include reading and writing).

Table 3: Scope for expert consensus biomarker recommendations.

- *Ready to be used in clinical trial* means that based on the available evidence it is recommended that the biomarker should be included in stroke clinical trials (pilot and feasibility work through to phase II/III/IV trials).
- *Development priority* refers to biomarkers where there is some evidence in human populations with stroke, but questions remain or the evidence is insufficient to support the inclusion of this biomarker in clinical trials at present. Predictive data of outcome and/or recovery is needed to establish its utility in clinical trials.

Table 4: Expert consensus biomarker recommendations

A) Motor

Ready to be used in clinical trial.

- CST indexed by DTI or by lesion overlap in the **hyperacute, acute, early and late subacute, and chronic phases**, which has demonstrated a moderate to strong relationship with impairment (outcome and recovery)
- TMS measure of MEP+ or MEP- of the upper limb to understand and track motor recovery up to the **late subacute phase** and understand the effects of rehabilitation interventions up to the **chronic phase** post-stroke. There is evidence of a strong relationship between impairment (outcome and recovery) and MEP status. We recommend that future studies of upper limb interventions determine whether patients are MEP+ or MEP- for the purposes of stratification.

Developmental priority

- Lesion location measured in the **hyperacute phase**. Identifying critical areas damaged that could predict recovery or treatment response may be important. Combining location and volume of stroke damage using multivariate techniques is the next logical step.
- Leukoaraiosis and covert lesions in the **hyperacute, early and late acute and chronic phases** require further validation to understand how they impact motor recovery.
- Accumulate further evidence of the usefulness of MEP+/- in the lower limb.
- Determining the utility of the laterality index from functional MRI as a predictor of efficacy in earlier stages post stroke is a developmental priority.
- Determine the utility of measures of rsFC and task-based activation and MEG/sEEG to predict treatment response in the **early subacute, late subacute and chronic stages** of recovery.

B) Somatosensory

Ready to be used in clinical trial

- There are no somatosensory system biomarkers ready for clinical trials.

Developmental priority

- Understand outcome and predict somatosensory recovery in the acute through to chronic stages using:
    - Biologically reliable measures of white matter fibre tract integrity and connectivity within brain networks.

- Measures of rsFC, and fMRI activation.

C) Cognition

Ready to be used in clinical trial

- There are no biomarkers ready to be used in clinical trials.

Developmental priority

- Understand outcome and predict recovery in the acute to chronic stages of recovery using
  - Measures of white matter integrity within both lesioned and non-lesioned areas.
  - Measures of rsFC and task-based FC.
- Predict treatment response in the early subacute, late subacute and chronic stages of recovery using measures of rsFC and fMRI activation.

D) Language

Ready to be used in clinical trials

- Index structural damage as per PLORAS imaging protocol in the chronic phase of recovery to understand outcome and predict recovery, which has demonstrated moderate accuracy.
- Index structural damage to and integrity of the arcuate fasciculus with diffusion weighted imaging in the late subacute and chronic phases of recovery to understand outcome, which has demonstrated a small to moderate relationship with language.

Developmental priority

- Predict recovery using perfusion CT and MRI in the hyperacute phase.
- Determine if structural damage predicts therapy or treatment response in the subacute through to chronic phases.
- Predict recovery and treatment response in subacute to chronic phase using measures of task-based fMRI activation.
- Explore measures of inter and intra-network connectivity and multivariate connectome-based symptom mapping to prediction of outcome and recovery.

Legend: CST, corticospinal tract; CT, computed tomography; DTI, diffusion tensor imaging; FC, functional connectivity; fMRI, functional magnetic resonance imaging; MEP+, motor evoked potential present; MEG, magnetoencephalography; MEP-, motor evoked potential absent; MRI, magnetic resonance imaging; PLORAS, Predicting Language Recovery and

Outcome After Stroke; rsFC, resting state functional connectivity; sEEG, sensory electroencephalography.