Harmonizing brain magnetic resonance imaging methods for vascular contributions to neurodegeneration

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Harmonizing Brain Magnetic Resonance Imaging Methods for Vascular Contributions to Neurodegeneration

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Abstract:	Introduction Many consequences of cerebrovascular disease are identifiable by magnetic resonance imaging (MRI), but variation in methods limits multicenter studies and pooling of data. The European Union Joint Programme on Neurodegenerative Diseases (JPND) funded the Harmonizing Brain Imaging Methods for Vascular Contributions to Neurodegeneration (HARNESS) initiative, with a focus on cerebral small vessel disease. Methods Surveys, teleconferences, and an in-person workshop were used to identify gaps in knowledge and to develop tools for harmonizing imaging and analysis. Results A framework for neuroimaging biomarker development was developed based on validating repeatability and reproducibility, biological principles, and feasibility of implementation. The status of current MRI biomarkers was reviewed. A website was created at www.harness-neuroimaging.org with acquisition protocols, a software database, rating scales and case report forms, and a deidentified MRI repository. Conclusions The HARNESS initiative provides resources to reduce variability in measurement in MRI studies of cerebral small vessel disease.

UNIVERSITY OF CALGARY
CUMMING SCHOOL OF MEDICINE

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Dear Dr. Snyder,

We are pleased to present our revised paper for consideration for publication in A & D: Diagnosis, Assessment, and Disease Monitoring.

Our manuscript is one of 10 papers that will be part of a Special Section on harmonization of neuroimaging methods in the context of neurodegenerative diseases.

Three additional working group members contributed to the manuscript revisions, whom we would like to add as coauthors: Dr. Walter Backes (Maastricht University Medical Center, Netherland; w.backes@mumc.nl), Dr. Michael Ingrisch (Ludwig-Maximilians-University Hospital Munich, Germany; michael.Ingrisch@med.uni-muenchen.de), and Dr. Stefan Ropele (Medical University of Graz, Austria; stefan.ropele@medunigraz.at).

Sincerely yours,

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DECLARATIONS OF INTEREST

The authors report no conflicts of interest.

ABSTRACT

Introduction: Many consequences of cerebrovascular disease are identifiable by magnetic resonance imaging (MRI), but variation in methods limits multicenter studies and pooling of data. The European Union Joint Programme on Neurodegenerative Diseases (JPND) funded the Harmonizing Brain Imaging Methods for Vascular Contributions to Neurodegeneration (HARNESS) initiative, with a focus on cerebral small vessel disease.

Methods: Surveys, teleconferences, and an in-person workshop were used to identify gaps in knowledge and to develop tools for harmonizing imaging and analysis.

Results: A framework for neuroimaging biomarker development was developed based on validating repeatability and reproducibility, biological principles, and feasibility of implementation. The status of current MRI biomarkers was reviewed. A website was created at www.harness-neuroimaging.org with acquisition protocols, a software database, rating scales and case report forms, and a deidentified MRI repository.

Conclusions: The HARNESS initiative provides resources to reduce variability in measurement in MRI studies of cerebral small vessel disease.

INTRODUCTION

Vascular disease contributes to more than half of dementia cases, often in conjunction with Alzheimer's disease pathology[1]. Most of the vascular brain injury is caused by cerebral small vessel disease (cSVD)[2], which often goes clinically unrecognized until revealed by brain imaging. cSVD is strongly associated with cognitive impairment and future risk for cognitive decline and dementia[3,4]. One of the challenging but intriguing aspects of research in this field is that cSVD has diverse manifestations, including brain infarcts, lacunes, white matter hyperintensity (WMH) of presumed vascular origin, perivascular spaces, and microbleeds[5].

Additionally, several promising new imaging biomarkers are emerging for the diagnosis and monitoring of patients, as well as for studies into etiology and pathophysiology[6,7].

The Standards for Reporting Vascular Changes on Neuroimaging (STRIVE)[5] were an important first step to harmonize neuroimaging assessment of cSVD. Terms and definitions for common cSVD lesion types, reporting standards, and suggestions for acquisition protocols were provided, and are now commonly used in research practice. However, STRIVE did not address pathways for developing and validating new biomarkers, nor did it address sources of variability in measurement, which should be minimized to enhance the ability to detect biological differences in multicenter and longitudinal studies.

To fully realize the potential of neuroimaging biomarkers of cSVD for use in larger scale, multicenter studies including clinical trials with cSVD endpoints, we created the Harmonizing Brain Imaging Methods for Vascular Contributions to Neurodegeneration (HARNESS) initiative. This initiative builds on the work of STRIVE by defining a framework for developing neuroimaging biomarkers of cSVD, reviewing the status of emerging neuroimaging biomarkers in this field, and developing and implementing standardized acquisition protocols and web-based repositories to facilitate multi-center research.

METHODS

HARNESS Group Composition

HARNESS was funded by the international Joint Programme for Neurodegenerative Diseases initiative to address neuroimaging biomarkers in neurodegeneration and dementia. The HARNESS members were invited to participate based on contributions cSVD research including their participation in STRIVE, and to provide a balance of input from different geographic regions and research disciplines. HARNESS included 70 members from 29

institutions in 11 countries, representing disciplines including radiology, biomedical engineering, clinical trials, computer science, epidemiology, medical biophysics, neurology, stroke medicine and psychiatry. Members were surveyed to identify important needs for harmonizing neuroimaging methods for cSVD, and then subdivided into 11 working groups of 6-12 participants representing a range of disciplines, cSVD interests and location, to address these needs. The initiative commenced in July 2016 and culminated in an in-person conference in June 2017. Where appropriate, working groups identified relevant papers through literature searches, expert knowledge, and hand searching articles from reference lists, but formal systematic reviews and creation of evidence tables were considered out of scope.

RESULTS

Neuroimaging Biomarker Framework for cSVD

We adopted the definition of a biomarker used by the Biomarkers Definitions Working Group[8]: "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". Inherent to this definition is that biomarkers may have different clinical purposes including diagnosis, prognosis, monitoring, and measuring treatment response. Biomarkers have been used as surrogate endpoints for clinical trials, meaning that the biomarker substitutes for or represents a manifestation of the clinical endpoint, when the biomarker is expected to predict "clinical benefit or harm based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence"[9]. This might be considered the highest level of qualification for a biomarker. However, biomarkers have other important uses for investigation, diagnosis, and monitoring of disease even if they do not predict treatment response.

Validation is required to determine whether a biomarker can be considered fit for a specific purpose. Some regulatory authorities, such as the U.S. Food and Drug Administration (FDA), define a formal process of biomarker qualification for use in evaluating therapeutics[10]. To our knowledge, no biomarker of cSVD, including WMH, lacunes, or microbleeds, has yet been submitted to and qualified by the US FDA for use in clinical trials, although they have been used as secondary endpoints in imaging substudies[11]. Qualification of an imaging marker that can be used as a trial endpoint would greatly accelerate the development of therapies for cSVD by improving selection criteria, reducing the size and cost of a trial and increasing the specificity of the outcome.

To facilitate validation of cSVD biomarkers we present a framework for neuroimaging biomarker development in Figure 1, adapted from consensus recommendations from the European Society of Radiology[12] and for development of imaging biomarkers for oncology[13]. Validation has technical aspects (e.g., can the same measurement be reproduced reliably on the same scanner or different scanners?), biological aspects (e.g., is the measurement different in patients with vs. without cSVD?), and feasibility of implementation (e.g., is the measurement practical and affordable?). In our version of this biomarker development framework, we define *proof of concept* as validation of measurement of a specific change or process (e.g., that arterial spin-labeling [ASL] MRI generates a signal that correlates with gold standard measurement of perfusion) while *proof of principle* refers to validation that the measurement distinguishes cases from controls or is associated with health outcomes (e.g., that ASL measured perfusion is different in cSVD patients than in controls and is associated with worse prognosis)[12]. We define *proof of effectiveness* as the ability to measure the marker across larger groups of patients at multiple sites[12]. *Repeatability* refers to the precision of repeated measurements under the same conditions using the same scanner (with high repeatability conferring greater power to detect smaller within-individual changes

over time, important for longitudinal studies), while *reproducibility* refers to the precision of replicate measurements on the same or similar objects (*e.g.* a phantom or human volunteers) using different scanners[12,13]. For visual assessments by human raters, *intra-rater reliability* refers to the precision of measurement by the same rater while *inter-rater reliability* refers to the precision of measurements across different raters. The Quantitative Imaging Biomarker Alliance (QIBA) offers recommendations for study design and statistical approaches to technical validation[14]. Validation typically begins with relatively small, cross-sectional studies at single centers to demonstrate proof of concept, proof of principle and initial technical validation, before expanding to longitudinal studies and multicenter studies to demonstrate proof of effectiveness and reproducibility. Feasibility is then demonstrated by incorporation of the biomarker into clinical radiological practice or by qualification for use in clinical trials.

Survey of Current cSVD Biomarker Development with Specific Considerations for Selected Emerging Modalities

Commonly studied neuroimaging biomarkers of cSVD are lacunes, WMH of presumed vascular origin, and cerebral microbleeds. These lesions are typically reported in routine radiology practice and have been incorporated as secondary imaging endpoints in some clinical trials. For these markers proof of concept, principle, and effectiveness have been established. Even so, longitudinal data on change over time and data on repeatability and reproducibility, so important for planning sample sizes in clinical trials, are relatively scant[15,16].

A recent systematic review highlighted the gaps in knowledge in repeatability and reproducibility of measurements of cSVD lesions, focusing mostly on quantitative biomarkers including volumes of WMH, lacunes, and brain[17]. The authors systematically

searched the literature to identify information on scan-rescan repeatability (which they termed "within center reproducibility") as well as the effects of scanner vendor, field strength, sequence choices, and coil type. They found that the amount of literature on repeatability and reproducibility varied widely by lesion type. The most literature was found on measures of brain volume, probably because brain atrophy is an important biomarker for many neurological diseases in addition to cSVD, such as Alzheimer's disease, and because phantoms are available for measuring variations in geometric distortions across scanners. For WMH, lacunes, perivascular spaces, and microbleeds there was only sparse information on repeatability with relatively speaking the greatest amount of information on WMH measurements cross-sectionally, but no repeatability data on longitudinal measurements.

Figure 2 provides an overview of the validation status of the best established cSVD markers as well as emerging modalities and techniques. Over time the list of neuroimaging biomarkers of cSVD has grown substantially as our knowledge of cSVD pathophysiology[2], and ability to image it, has grown.

Some markers have already received a large amount of attention, notably WMH (assessed visually or computationally), lacunes, and microbleeds (mainly visually with some emerging computational methods). Even so, some aspects of validation are lacking with few large comparisons of different volumetric tools, little longitudinal data, and none are yet adopted as confirmed surrogate outcomes in clinical trials. Nonetheless, they have already been the subject of many reviews[16,17].

Hence, the list of biomarkers discussed in detail here represents the subset that the HARNESS group selected as the next most promising for measuring unique aspects of cSVD pathophysiology, but that have so far received less attention. The list is not exhaustive. Future research will likely add more modalities and lesion types. For example, microinfarcts have been visualized on MRI by several research groups and may be a frequent but

underrecognized consequence of thrombosis or embolism of small arteries[18]. Additionally, future research may clarify that biomarkers currently on the list are a poor fit for some purposes.

In the following sections, we review the state of imaging biomarker development for selected emerging modalities, along with considerations for further development and harmonization.

Structural Imaging: Perivascular spaces

Perivascular spaces are rapidly emerging as a novel marker of cSVD and are defined as "fluid-filled spaces that follow the typical course of a vessel as it goes through grey or white matter"[5]. While long considered an innocuous phenomenon of aging, a converging body of proof of principle cross-sectional studies now suggests that a larger burden of perivascular spaces is associated with a higher likelihood of dementia, cognitive impairment, and stroke[19-21] More importantly, these associations are independent from established markers of cSVD. Longitudinal studies of the appearance of perivascular spaces or their enlargement over time are lacking; therefore, the rate at which these spaces change over time is essentially unknown. One study showed that the 5-year incidence of new large perivascular spaces (defined as ≥3 mm diameter) in a general elderly population was 3.1%[21], however this size exceeds the generally accepted current width boundary between perivascular spaces and lacunes[5].

There are few data on the repeatability of measurements of perivascular spaces and reproducibility of measurement across scanners. For one automated method, repeatability was excellent with intra-class correlations of 0.92 for basal ganglia and 0.87 for centrum semiovale[22]. In contrast, intra-rater and inter-rater reliability for visual rating scales have been published by several groups and should be expected to be good to excellent (i.e., with kappa values of 0.5 or higher or intra-class correlation coefficients of 0.6 or higher). Rating on T2-weighted sequences is favoured because perivascular spaces are well visible, but some

studies have used high resolution T1-weighted sequences instead. In one study, ratings on T1-weighted and T2-weighted sequences showed excellent correlation (intraclass correlation >0.80)[23].

The HARNESS working group identified several difficulties in the quantification of perivascular spaces, which have so far hampered comprehensive understanding of their biological meaning. First, perivascular spaces, reflecting the virtual space between blood vessels and pia mater, by themselves are a physiologic finding. It is the enlargement of these spaces that can be visible on MRI that is considered non-physiologic. The question then remains what amount of enlargement should distinguish physiologic from non-physiologic perivascular spaces? Originally, a convenience threshold was chosen, such that any perivascular space visible on brain MRI was considered enlarged. However, increasing field strengths and other advances in imaging now allow much smaller perivascular spaces to become visible on MRI, indicating the need to use a more objective and reproducible threshold independent from imaging parameters.

Second, since perivascular spaces are defined by their intricate relation to brain vessels, they are ubiquitous in all brain regions. Yet, the extent of enlargement is different across brain regions and should be taken into account in their quantification. A working upper width limit of 3 mm is widely used to discriminate perivascular spaces from small lacunes[5], but for example it is well recognized that larger width perivascular spaces are sometimes seen in the substantia innominata. Radio-pathological correlation studies show that MRI can differentiate perivascular spaces from lacunes with good sensitivity and specificity using morphological and signal intensity information[24], but more validation on correlations by region would be welcome. Similarly, the processes underlying their enlargement are thought to differ according to brain region; for example, in cerebral amyloid

angiopathy enlargement of perivascular spaces is seen in the centrum semiovale but not in the basal ganglia.[25,26].

Against this background, it is not surprising that the various efforts to quantify perivascular spaces have differed with respect to definition of enlargement, regions to be scored, and scoring system used[23,27-30]. While work continues to identify the key features of these rating systems with respect to similarities, dissimilarities, strengths, weaknesses, and 'translation' from one rating system to the other, we recommend that investigators use the rating system most relevant to their population, or that they are most comfortable with, while having a core understanding how that specific rating system relates to others available in the literature. Raters should be trained on a standardized dataset with measurement of intra-rater and inter-rater reliability and report these measures in publications; training tools are available on HARNESS website.

Parallel to this development of visual rating, there is now a strong focus on fully-automated quantification of perivascular spaces. These efforts have so far been hampered by similar methodological considerations as outlined above, but the recent introduction of machine learning algorithms in brain imaging holds great promise in overcoming these barriers[22,31]. Just like automated quantification of WMH resulted in dramatic improvement in our understanding of their role in neurodegenerative diseases particularly at the voxel level, automated detection, volumetrics, shape, density and orientation of perivascular spaces could signify a paradigm shift in their position within the pantheon of cSVD markers.

Structural Imaging: Atrophy in the context of cSVD

Atrophy is now a well-established, measurable consequence of cSVD. Both cross-sectional and longitudinal studies show proof of principle that total brain volume is lower in cSVD and decreases more quickly in persons with enlarging WMH. The repeatability and reproducibility of brain volume measurements in the context of cSVD has been reviewed

recently[17]. Here, we highlight specific aspects to be considered when implementing atrophy measurements in cSVD studies.

Given the complexity of brain anatomy, measures of brain volume should be obtained from 3D T1-weighted high-resolution isotropic sequences with quantitative computerized methods where possible. To capture chronic, final effects, the image acquisitions should be performed remotely in time (probably 90 days or longer) from the occurrence of acute brain lesions.

At a given time point, volumetric measures reflect the sum of the individual's maximum brain volume growth (estimated by the intracranial cavity volume), the effect of age, and that of multiple potential diseases including cSVD, overt stroke, and neurodegenerative diseases such as Alzheimer's disease. Controlling for differences in head size, e.g. by expressing volumes as a fraction of intracranial volume or including intracranial volume as a covariate, is mandatory in single time point analyses. Although controlling for intracranial volume is not strictly necessary for longitudinal analyses, investigators may still want to analyze it as a proxy for original maximum brain size which reflects premorbid brain health and is associated with general intelligence[32]. In longitudinal analyses, the use of cross-timepoint registration pipelines rather than repeated use of cross-sectional methods may reduce variability in measurement[33,34] but the optimal approach remains to be confirmed.

Methods involving registration to a common template should be used cautiously given that brains with cSVD, often exhibiting large ventricles and white matter atrophy, can register poorly to atlases based on healthy individuals. This is a particularly challenging problem when cSVD is accompanied by larger destructive intracerebral hemorrhages or infarcts. The impact of brain tissue lesions on the different methods to assess brain volume is often unpredictable[35]. In particular, the presence of extensive WMH can lead to erratic behavior of most algorithms,[36,37] and if appropriate they should be masked. Additionally, algorithms may

variably segment fluid-filled cavities within the brain (lacunes and enlarged perivascular spaces) as cerebrospinal fluid, gray matter or white matter, requiring a systematic visual quality control of segmentation results[35,38]. There is consensus that cavities resulting from infarction should be excluded from brain tissue estimates[5], depending on the question being asked; clearly, they do not represent spaces such as subarachnoid space or ventricles but nor do they represent normal brain tissue. They can be considered as part of the 'total burden of brain injury'[39] in some analyses. Quantitative methods are emerging that can estimate perivascular space volume; when such measurements are made we recommend that perivascular space volume be reported as a separate tissue class and not included in the total brain volume. Given the numerous sources of variation in gray to white contrast in cSVD, differential measures of gray and white matter volumes should be interpreted carefully[40]. The use of other computational volumetric markers, such as ventricle volumes, has not been validated in cSVD. All methods require visual checking and may need manual editing where automated segmentation has failed to identify the correct tissue.

Diffusion imaging metrics

Diffusion imaging provides of the diffusion of water molecules within brain tissue. There are a large variety of techniques to analyze these data. Diffusion-weighted imaging is positive (that is, shows increased signal) in the setting of recent infarction or microinfarction. Scalar measures describe diffusion properties on the voxel level, such as the extent or directionality. Diffusion tensor imaging (DTI) is the most useful model to derive these scalar metrics such as mean diffusivity (MD) or fractional anisotropy (FA). Tractography can be used to visualize fiber connections and analyze diffusion on the tract level. Global tractography in combination with graph theoretical network analysis allows to assess the impact of cSVD on the level of brain networks.

Proof of principle that diffusion imaging metrics can serve as biomarkers of cSVD is well established by multiple studies associating diffusion imaging indices derived from the white matter (WM) or normal-appearing WM (NAWM) with cSVD and cSVD risk factors. Most studies report cross-sectional associations between lower FA or higher MD and cognitive and gait impairments[41,42] Mean diffusivity is readily measured in the whole brain, tissue subregions, regions of interest or tracts and shows the strongest associations with SVD lesion burden[43]. Recent, promising post-processing methods to increase the reliability and ease of extraction of diffusion imaging metrics include histogram-derived diffusion imaging metrics, such as the peak width of the skeletonized MD distribution (PSMD)[44], and connectivity measures including ones based on network theory[45-47]. Lower brain connectivity in strategic network locations, such as long-distance fibers connecting so-called network 'hubs', show promise for prediction of speed and executive functioning[48,49]. This is not an exhaustive list, as there are several other promising diffusion imaging acquisition and analysis methods which show promise for development as biomarkers of cSVD[50,51].

In contrast to the many cross-sectional studies, there are fewer studies evaluating diffusion imaging as a prognostic marker of disease progression.[41] The LADIS study reported an association between NAWM MD at baseline and decline in processing speed,[52] whereas the RUN DMC study found no association between baseline NAWM MD and cognitive decline[53], or risk of dementia over 5 years[54]. Diffusion imaging-derived brain connectivity predicted conversion to dementia after 5 years[55]. Longitudinal studies of diffusion imaging change over time are at this time relatively scarce[56-60] but promising, suggesting that change over time can be detected on diffusion imaging with similar sensitivity as change over time in WMH volume, requiring smaller sample sizes than required to detect atrophy or incident lacunes[61]. Progression over time in diffusion imaging metrics has been associated with increased risk of dementia[58] and gait decline[62].

The tissue correlate of altered diffusion metrics in cSVD is still debated. A recent study suggests that increased extracellular water content is a major contributor[50].

There are few studies on repeatability and reproducibility. The only study in patients with cSVD showed high reproducibility of PSMD in 7 patients with CADASIL scanned on a 1.5T and 3T scanner (intraclass correlation 0.95)[44]. Other studies in healthy controls have shown good repeatability and reproducibility for FA and MD measurements (coefficient of variation ranging from 0.8 to 5.7%)[63-65]. Nonetheless, variation in scanner or scanner upgrades may bias measurements in longitudinal studies[63]; therefore, investigators ideally should avoid scanner upgrades or changing scanners between baseline and follow-up measurements in studies designed to detect small changes over time. Phantoms to estimate reproducibility are in development.[66]

Perfusion and cerebrovascular reactivity

Perfusion and cerebrovascular reactivity (CVR) approaches are highly relevant in cSVD research because reduced tissue perfusion and impaired CVR are hallmark pathological features. These physiological forms of imaging introduce a unique set of challenges for study design, given the large variability in acquisition methods for perfusion and especially CVR which are less well established compared to many structural imaging techniques. To image CVR, the investigator must choose among several experimental methods for stimulating changes in cerebral blood flow (CBF), as well as between several different acquisition types such blood oxygen level dependent (BOLD) or arterial spin labeling (ASL). Because the vascular signal comes from only a proportion of voxel contents (the blood volume fraction in grey matter accounts for 5 to 10% of the tissue volume), and for BOLD-related techniques the changes in hemoglobin oxygenation are relatively small, attention must be paid to ensure sufficient signal to noise ratio to generate images of adequate quality.

Dynamic susceptibility contrast (DSC) and ASL are examples of MRI acquisitions that yield perfusion-weighted images; the former relies on an exogenous gadolinium contrast agent, while the latter uses magnetically labeled arterial blood water that is proximal to the imaging volume to label blood and produces quantitative perfusion maps typically expressed in units of mL/100g tissue/minute.

ASL is a promising modality for repeated measure studies because it does not require administration of an exogenous intravenous contrast agent. A fraction of cSVD articles on perfusion have thus far used ASL[67]; cross-sectional studies, for example, provide proof of principle by showing that a pattern of reduced frontal perfusion was associated with increased WMH volume[68]. Longitudinal studies are less common, however, one 4-year follow-up study reported that global CBF decreases were associated with higher baseline WMH but that baseline CBF was not associated with greater WMH progression.[69] Another longitudinal study found that while lower baseline CBF predicted appearance of new WMH at 18 months, change in CBF was not associated with new WMH[70]. Studies are needed on the association of baseline and longitudinal CBF and the prevalence and incidence of new brain infarcts and microinfarcts. Although white matter and subcortical tissue perfusion estimates are of particular interest in cSVD, these measurements are less robust than in grey matter when using ASL[71] due to the lower CBF and longer arterial transit time.

A validation study of ASL found higher repeatability for pseudo-continuous ASL compared with pulsed ASL or continuous ASL, with a coefficient of variation of 3.5% in gray matter and 8.0% in white matter[72]. There are few reproducibility studies across scanner types. One study found high reproducibility in eight volunteers scanned on two General Electric (GE) 3T scanners[73]. Another study found that sequence parameter differences had a larger effect than hardware or software differences on General Electric, Philips, and Siemens scanners[74]. Phantoms for ASL have been developed but not yet widely adopted[75].

Unlike physiological imaging during a single "baseline" state, CVR involves physiological provocation to measure a vasoactive response, typically by breathing medical air enriched with carbon dioxide gas. Technical and paradigm details and considerations have been recently reviewed[76]. Multi-contrast physiological imaging, combining perfusion and CVR maps in cSVD, is a promising technique[77]. At this time, relatively few CVR studies have focused explicitly on cSVD[78]. However, CVR imaging is being exploited as an imaging endpoint to assess the efficacy of vasodilatory drugs in a dose escalation trial[79]. CVR appears to be a promising prognostic biomarker of cSVD brain changes, for example as revealed by one longitudinal study that found impaired regional CVR was predictive of WMH lesion expansion at one-year follow-up[80]. A four-year longitudinal study showed that age-related decreases in CVR were associated with steeper declines in processing speed and episodic memory but not working memory or reasoning; however, the degree to which enlarging WMH or new infarction may have been associated with these changes was not assessed. The BOLD-response to a visual stimulus has been shown to be a possible biomarker for CAA and could be a more easily implemented, well-tolerated alternative means to measure CVR, but is is limited to the occipital lobe[81-83] and has not been compared directly to CVR measurement based on hypercapnia.

The repeatability of CVR measurements has been investigated in healthy controls but not patients with cSVD. In a study of 15 controls, the coefficient of variation ranged from 7.3% to 42.9% across 16 regions of interest including cortical and subcortical grey matter and white matter[84]. The coefficient of variation was lower when using a paradigm that averaged two three-minute blocks of CO_2 inhalation rather than three one-minute blocks[84].

A consensus group has provided recommendations for ASL imaging protocols[85]; however, long-label and long-delay ASL approaches may prove superior for CBF measurement in the white matter and subcortical gray matter. Multicenter studies using

scanners from different vendors seems justifiable as long as key methods (including choice of pseudo-continuous ASL, readout strategy, labeling duration, and post-labeling delay time) are kept constant. For CVR imaging, there are a greater diversity of methods and the different methods may suit specific patient populations. One published protocol[84] using three-minute CO_2 blocks is being used in a multicenter trial.

Blood-brain barrier integrity

Although proof-of-concept evidence is very limited, proof-of-principle evidence from cross-sectional clinical studies suggests that blood-brain barrier (BBB) dysfunction determined by MR is associated with imaging features of cSVD, and that BBB leakage may contribute to tissue damage, development of cSVD features and long-term adverse outcomes[86,87]. Therefore, BBB permeability is an important target of measurement in studies of pathophysiology and treatment evaluation.

Dynamic contrast-enhanced MRI (DCE-MRI) using a standard dose of a gadolinium-based contrast agent is presently the most promising technique for quantitative imaging of subtle leakage[86], and has been applied in several studies of cSVD and related conditions.[86,88-91] However, while the technique is well-established in other conditions such as brain tumours, particular challenges emerge in cSVD due to the slow rate of leakage. For qualitative assessment, gadolinium-based contrast agent (GBCA) enhancement of cerebrospinal fluid on T2-weighted fluid attenuated inversion recovery (FLAIR) and T1-weighted imaging may provide a practical, though non-specific, alternative[92,93]. Other potential methods are difficult to quantify (e.g. dynamic susceptibility contrast MRI),[94] employ ionising radiation,[95,96] or are at an early stage of development (compartmental ASL modelling[97-99]). Nevertheless, DCE-MRI is not routinely used in cSVD studies due to practical impediments (long scan time, exogenous contrast), lack of widespread expertise, and technical and physiological complexities and confounds[100,101].

There are few studies of BBB permeability change over time in cSVD. A single study of 22 subjects with high WMH burden reported little overlap between regions of high white matter permeability between the first and second scan, but that high permeability was often seen along the border of WMH at either time[102].

Because there is no reliable convenient reference method for quantifying subtle BBB permeability, studies comparing DCE-MRI measurements with other measures of BBB integrity are few and inconclusive[103,104]. The need for a second gadolinium administration is a barrier to conducting studies on repeatability, but one study showed good evidence of repeatability with coefficient of variation of 11.6 % for white matter and 14.4 % for gray matter at 3T[105]. Reproducibility across different MR hardware has not been investigated. Based on theoretical considerations and experimental observations, it is likely that measurements are influenced by MR field strength, scanner stability, spatial resolution, pulse sequence parameters, acquisition time, GBCA type, and pharmacokinetic model[100,101,106,107]. The diversity of acquisition and analysis protocols described (sometimes incompletely) in the literature is, therefore, a key impediment to the interpretation and comparison of data from different studies and centres.

Our recommendation for future studies is to use a three-dimensional, MR acquisition with wide spatial coverage, pre-contrast T1 measurement, a minimum temporal resolution of around one minute and minimum DCE scan time of 15 minutes[108]. A vascular input function should be measured in the venous sinuses and the permeability-surface area product *PS* for tissue regions or, where feasible, individual voxels should be estimated using an appropriate pharmacokinetic model, typically the Patlak model[109]; simulations may be performed to assess accuracy and precision. Results should be interpreted carefully, particularly when comparing data from different research groups or scanners. We identify three priorities for the development of this biomarker: (i) agreement by the wider cSVD and dementia imaging

research community on an open-access, dynamic consensus protocol for DCE-MRI measurements of slow BBB leakage, (ii) acquisition of data on repeatability and reproducibility, and (iii) studies to assess accuracy, including theoretical work, comparison with independent measures of BBB integrity, and validation using MR test objects and histology. Further technical development to increase accuracy and precision, as well as continued development of alternative methods are also encouraged.

Ultra high field MRI

Ultra-high field MRI, in particular 7T MRI, is emerging as a new tool in cSVD research. The higher resolution, different tissue contrasts, and better signal to noise ratios of 7T MRI allow the investigator to probe aspects of cSVD that are difficult to assess at lower field strength. In addition to enhanced sensitivity for cSVD lesions such as microinfarcts and microbleeds and more precise assessment of atrophy[18,110], with 7T MRI it is possible to actually visualize the small vessels[111]. From both perforating arteries and veins features such as vessel density, length, and tortuosity can be resolved.[111,112]. Additionally, different aspects of vascular function, including blood flow, pulsatility of flow in small penetrating arteries (a possible indicator of vascular stiffness), vascular reactivity to vasoactive agents (e.g carbon dioxide) or neuronal stimulation (i.e. functional MRI), can be assessed, making it possible to probe cSVD at the level of the small vessels themselves[111].

Despite the potential for of 7T MRI for cSVD, important steps have to be taken to validate these novel techniques. Of note, EUFIND (the European Ultrahigh-Field Imaging Network in Neurodegenerative Diseases), another JPND initiative, has the goal of harmonizing 7T MRI protocols across more than 20 centres from Europe and the US.

Tools to Facilitate cSVD Biomarker Development and Harmonization

The HARNESS initiative focused on three areas to provide tools for harmonization: MR acquisition, post-processing, and common repositories for training and validation. These tools are made available to the research community at www.harness-neuroimaging.org.

The HARNESS website provides fully specified MR acquisition protocols suitable for research studies that include a focus on cSVD. Given the diversity of manifestations of cSVD and hypotheses that can be tested, there is no single MR acquisition protocol that can quantify all aspects of cSVD and therefore investigators must make choices regarding protocol composition, also accounting for issues of feasibility including acquisition time and cost. Therefore, instead of a single protocol the HARNESS website provides several options that meet these criteria: a) they adhere to STRIVE[5], b) they are suitable for identifying canonical cSVD lesions types--lacunes and WMH of presumed vascular origin, recent small infarcts, microbleeds, atrophy, and DTI changes, c) they have been tested on more than one scanner as part of an established multicenter study and d) the protocol developers are willing to share the protocol freely. There are also links to other websites and useful repositories of information.

Currently, protocols are available from the SVD@target study[84] (ISRCTN10514229) and the Canadian Dementia Imaging Protocol[113], with plans to add the protocol from the U.S. National Institute of Neurological Disorders and Stroke MarkVCID Biomarker Consortium (https://markvcid.partners.org/) once it has been fully specified and tested. Sequence parameters with exam cards are provided for 3T for most of the major vendors including General Electric, Phillips, and Siemens. The protocols are suitable for prospective research studies with quantitative imaging biomarkers but probably exceed most clinical stroke protocols in terms of acquisition time, spatial resolution, and inclusion of DTI. They have been implemented successfully in multicenter studies at research sites, but nonetheless may not be feasible for multicenter studies performed at predominantly clinical scan sites where the intent is to leverage clinical imaging without a focus on quantitative biomarkers.

Reducing imaging variability may be enhanced by following consensus recommendations[17] to perform automated quality checks for acquisition parameters and monitoring of images for artefacts, correction for gradient nonlinearities, a well-defined method for subject's positioning in the scanner, and a clear strategy for hardware replacement when needed.

The HARNESS software database provides a searchable source for information on downloadable software tools for processing MR data for cSVD quantitative biomarkers, such as for segmenting WMH. There are many existing software libraries for neuroimaging analysis, but only HARNESS focuses exclusively on cSVD. Site users can search for software by image modality, measurement type, key words, availability (i.e. by download or by request to the developer), or operating system. Software developers control their own entries via password-protected accounts, and must make their software available according to their own terms by providing a link or through contacting the developer. We are actively recruiting developers with tools to sell or share. Developers may access the site for information on how to create accounts.

To aid visual review for cSVD lesions according to STRIVE, the HARNESS site makes downloadable electronic documents available including validated visual rating scale scores and instructions, case report forms, and training slides.

Training readers and software algorithms requires access to independent MR datasets for measurements. The HARNESS site includes a web-based repository with completely de-identified 3T MR data showing lacunes, WMH, microbleeds, and cortical superficial siderosis from patients with TIA, minor ischemic stroke, and cerebral amyloid angiopathy, with consensus "gold standard" measurements for comparison. This repository will be useful for independently confirming reliability of measurements within and across research groups, and for derivation and validation of computerized algorithms for quantitative measurement

(e.g. for segmenting WMH to determine location and overall volume) as well for comparing WMH algorithms against an independent standard.

SUMMARY AND CONCLUSIONS

The HARNESS initiative was a multidisciplinary consensus process with input from a large number of neuroimaging researchers investigating cSVD. Our group developed a framework for neuroimaging biomarker development closely aligned with those proposed in other areas of imaging research. The HARNESS website (www.harness-neuroimaging.org) was created to facilitate harmonized neuroimaging methods for cSVD research. The site includes cSVD-appropriate MR acquisition protocols aligned with STRIVE, a searchable database of softwares for analyzing brains with cSVD, visual rating scales and case report forms, and a repository of 100 deidentified scans demonstrating different cSVD lesion types. These tools and resources are made available to the research community via the site and can be easily updated by contributors.

In this rapidly evolving field, we found that the degree of biomarker validation—technical, biological and clinical, and feasibility—varied by cSVD lesion and measurement type. In general, visually diagnosed cSVD lesions such as lacunes, WMH, and microbleeds have the greatest amount of clinical validation including as prognostic markers and data are available on incidence andchange over time, and are already being used in multicenter studies and reported in routine clinical practice. Even so, none of these markers has yet been qualified for use in clinical trials by regulatory agencies, and more work is needed to standardize and compare current volumetric tools. Other markers are at a less advanced stage of biomarker development. Atrophy has been extensively studied but almost always in the context of Alzheimer's disease and not cSVD. Among the emerging cSVD markers there are relatively more data on diffusion imaging and perivascular space imaging, but more

longitudinal data and multicenter data on reproducibility are needed. Measurements of brain perfusion, vascular reactivity, and blood-brain barrier integrity are promising but are at an even earlier stage of development. For these cSVD manifestations innovation to overcome technical and feasibility barriers, rather than harmonizing to a best protocol, is the most important next step in development.

We found that technical validation often lagged clinical validation. However, estimates of repeatability and reproducibility are critically important to estimate minimum detectable differences over time and variability in measurement in multicenter studies, essential for sample size calculations for multicenter longitudinal trials. This lag in technical validation likely reflects the difficulty in obtaining funding for technical studies compared to clinical studies, the burden on research subjects to undergo multiple scans, and the general lack of non-human phantoms for studies of reproducibility. In contrast to volumetric imaging and functional MRI, phantoms for other measurements are less well developed. One research group has developed a phantom for iron deposits that mimic mineral deposits and microbleeds, not currently available for purchase[114]; otherwise, we are not aware of any other phantoms that recreate specific aspects of cSVD. Technical validation for neuroimaging biomarkers of cSVD would be enhanced by creating funding opportunities specifically for this purpose.

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Figure 1. Imaging Biomarker Development Framework for Cerebral Small Vessel Disease

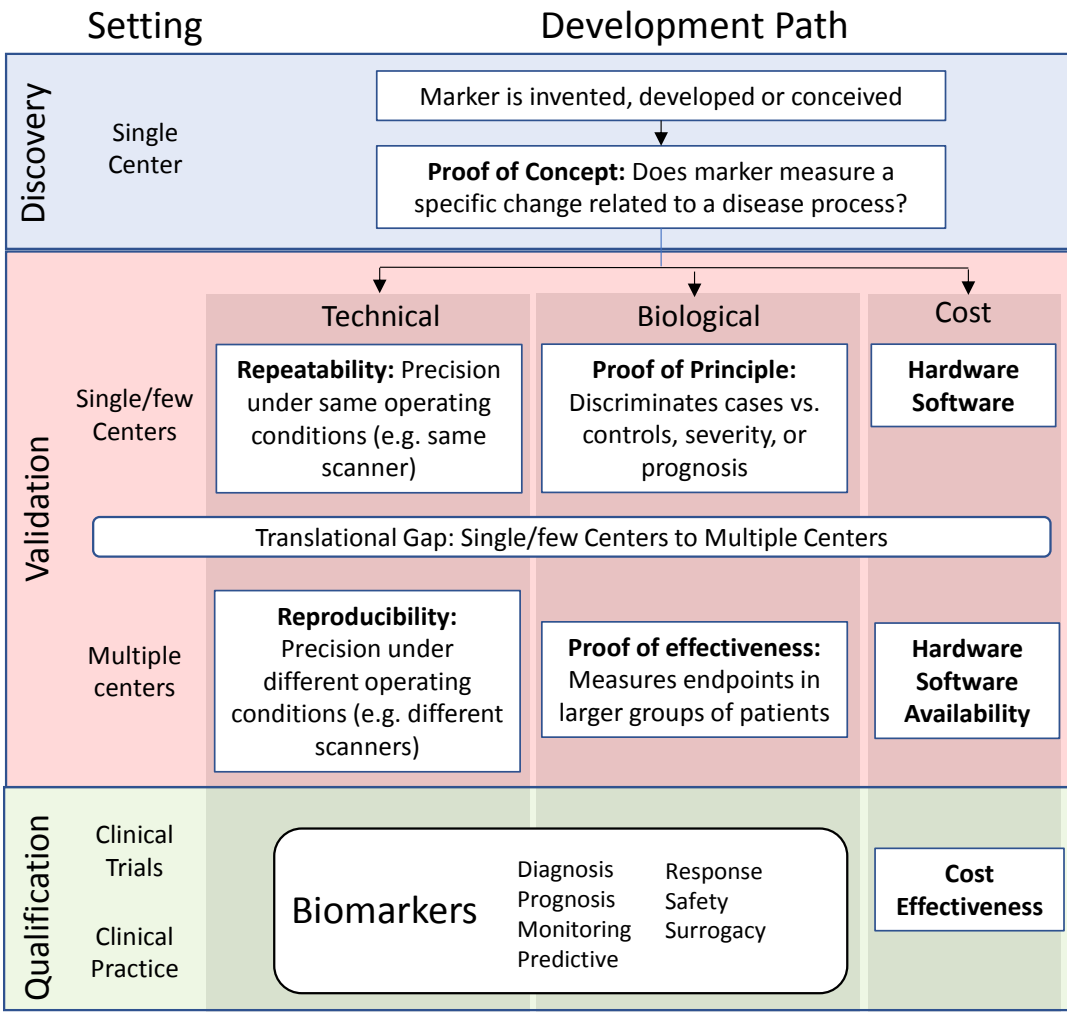

Figure 2. Schematic overview of neuroimaging biomarker development status for cerebral small vessel disease

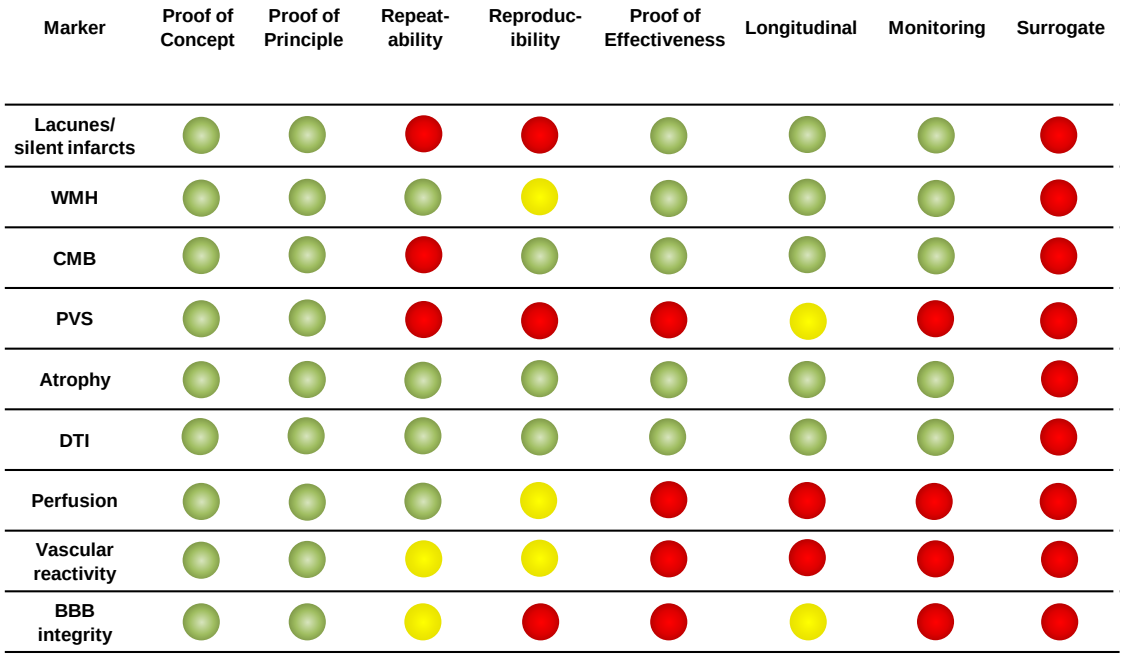

Figure 2 Legend: Green light indicates validation data from two or more studies from independent research groups; Yellow light indicates support from a single study or conflicting evidence from multiple studies; Red light indicates there is currently insufficient evidence. WMH, white matter hyperintensities of presumed vascular origin; CMB, cerebral microbleeds; PVS, perivascular spaces; DTI, diffusion tensor imaging; BBB, blood-brain barrier. *Proof of concept*: evidence that the marker measures a specific change or process related to cerebral small vessel disease. *Proof of principle/ Mechanism*: evidence that the marker differs between patients with and without cerebral small vessel disease. *Proof of effectiveness*: evidence from larger scale multiple center studies that the marker differs between patients with and without cerebral small vessel disease. *Repeatability:* precision of repeated measurements under the same conditions using the same scanner. *Reproducibility:* replicate measurements on the same or similar objects (e.g. a phantom or human volunteers) in different locations using different scanners. *Longitudinal:* rate of change over time has

been defined. *Monitoring:* evidence that longitudinal change in the marker is associated with progression of cerebral small vessel disease. *Surrogate*: evidence that change in the marker is strongly associated with clinical outcomes in cerebral small vessel disease, such that changes in the marker could be considered a substitute for a clinical endpoint.

Title: Harmonizing Brain Magnetic Resonance Imaging Methods for Vascular Contributions to Neurodegeneration

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DECLARATIONS OF INTEREST

The authors report no conflicts of interest.

ABSTRACT

Introduction: Many consequences of cerebrovascular disease are identifiable by magnetic resonance imaging (MRI), but variation in methods limits multicenter studies and pooling of data. The European Union Joint Programme on Neurodegenerative Diseases (JPND) funded the Harmonizing Brain Imaging Methods for Vascular Contributions to Neurodegeneration (HARNESS) initiative, with a focus on cerebral small vessel disease.

Methods: Surveys, teleconferences, and an in-person workshop were used to identify gaps in knowledge and to develop tools for harmonizing imaging and analysis.

Results: A framework for neuroimaging biomarker development was developed based on validating repeatability and reproducibility, biological principles, and feasibility of implementation. The status of current MRI biomarkers was reviewed. A website was created at www.harness-neuroimaging.org with acquisition protocols, a software database, rating scales and case report forms, and a deidentified MRI repository.

Conclusions: The HARNESS initiative provides resources to reduce variability in measurement in MRI studies of cerebral small vessel disease.

INTRODUCTION

Vascular disease contributes to more than half of dementia cases, often in conjunction with Alzheimer's disease pathology[1]. Most of the vascular brain injury is caused by cerebral small vessel disease (cSVD)[2], which often goes clinically unrecognized until revealed by brain imaging. cSVD is strongly associated with cognitive impairment and future risk for cognitive decline and dementia[3,4]. One of the challenging but intriguing aspects of research in this field is that cSVD has diverse manifestations, including brain infarcts, lacunes, white matter hyperintensity (WMH) of presumed vascular origin, perivascular spaces, and microbleeds[5].

Additionally, several promising new imaging biomarkers are emerging for the diagnosis and monitoring of patients, as well as for studies into etiology and pathophysiology[6,7].

The Standards for Reporting Vascular Changes on Neuroimaging (STRIVE)[5] were an important first step to harmonize neuroimaging assessment of cSVD. Terms and definitions for common cSVD lesion types, reporting standards, and suggestions for acquisition protocols were provided, and are now commonly used in research practice. However, STRIVE did not address pathways for developing and validating new biomarkers, nor did it address sources of variability in measurement, which should be minimized to enhance the ability to detect biological differences in multicenter and longitudinal studies.

To fully realize the potential of neuroimaging biomarkers of cSVD for use in larger scale, multicenter studies including clinical trials with cSVD endpoints, we created the Harmonizing Brain Imaging Methods for Vascular Contributions to Neurodegeneration (HARNESS) initiative. This initiative builds on the work of STRIVE by defining a framework for developing neuroimaging biomarkers of cSVD, reviewing the status of emerging neuroimaging biomarkers in this field, and developing and implementing standardized acquisition protocols and web-based repositories to facilitate multi-center research.

METHODS

HARNESS Group Composition

HARNESS was funded by the international Joint Programme for Neurodegenerative Diseases initiative to address neuroimaging biomarkers in neurodegeneration and dementia. The HARNESS members were invited to participate based on contributions cSVD research including their participation in STRIVE, and to provide a balance of input from different geographic regions and research disciplines. HARNESS included 70 members from 29

institutions in 11 countries, representing disciplines including radiology, biomedical engineering, clinical trials, computer science, epidemiology, medical biophysics, neurology, stroke medicine and psychiatry. Members were surveyed to identify important needs for harmonizing neuroimaging methods for cSVD, and then subdivided into 11 working groups of 6-12 participants representing a range of disciplines, cSVD interests and location, to address these needs. The initiative commenced in July 2016 and culminated in an in-person conference in June 2017. Where appropriate, working groups identified relevant papers through literature searches, expert knowledge, and hand searching articles from reference lists, but formal systematic reviews and creation of evidence tables were considered out of scope.

RESULTS

Neuroimaging Biomarker Framework for cSVD

We adopted the definition of a biomarker used by the Biomarkers Definitions Working Group[8]: "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". Inherent to this definition is that biomarkers may have different clinical purposes including diagnosis, prognosis, monitoring, and measuring treatment response. Biomarkers have been used as surrogate endpoints for clinical trials, meaning that the biomarker substitutes for or represents a manifestation of the clinical endpoint, when the biomarker is expected to predict "clinical benefit or harm based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence"[9]. This might be considered the highest level of qualification for a biomarker. However, biomarkers have other important uses for investigation, diagnosis, and monitoring of disease even if they do not predict treatment response.

Validation is required to determine whether a biomarker can be considered fit for a specific purpose. Some regulatory authorities, such as the U.S. Food and Drug Administration (FDA), define a formal process of biomarker qualification for use in evaluating therapeutics[10]. To our knowledge, no biomarker of cSVD, including WMH, lacunes, or microbleeds, has yet been submitted to and qualified by the US FDA for use in clinical trials, although they have been used as secondary endpoints in imaging substudies[11]. Qualification of an imaging marker that can be used as a trial endpoint would greatly accelerate the development of therapies for cSVD by improving selection criteria, reducing the size and cost of a trial and increasing the specificity of the outcome.

To facilitate validation of cSVD biomarkers we present a framework for neuroimaging biomarker development in Figure 1, adapted from consensus recommendations from the European Society of Radiology[12] and for development of imaging biomarkers for oncology[13]. Validation has technical aspects (e.g., can the same measurement be reproduced reliably on the same scanner or different scanners?), biological aspects (e.g., is the measurement different in patients with vs. without cSVD?), and feasibility of implementation (e.g., is the measurement practical and affordable?). In our version of this biomarker development framework, we define *proof of concept* as validation of measurement of a specific change or process (e.g., that arterial spin-labeling [ASL] MRI generates a signal that correlates with gold standard measurement of perfusion) while *proof of principle* refers to validation that the measurement distinguishes cases from controls or is associated with health outcomes (e.g., that ASL measured perfusion is different in cSVD patients than in controls and is associated with worse prognosis)[12]. We define *proof of effectiveness* as the ability to measure the marker across larger groups of patients at multiple sites[12]. *Repeatability* refers to the precision of repeated measurements under the same conditions using the same scanner (with high repeatability conferring greater power to detect smaller within-individual changes

over time, important for longitudinal studies), while *reproducibility* refers to the precision of replicate measurements on the same or similar objects (*e.g.* a phantom or human volunteers) using different scanners[12,13]. For visual assessments by human raters, *intra-rater reliability* refers to the precision of measurement by the same rater while *inter-rater reliability* refers to the precision of measurements across different raters. The Quantitative Imaging Biomarker Alliance (QIBA) offers recommendations for study design and statistical approaches to technical validation[14]. Validation typically begins with relatively small, cross-sectional studies at single centers to demonstrate proof of concept, proof of principle and initial technical validation, before expanding to longitudinal studies and multicenter studies to demonstrate proof of effectiveness and reproducibility. Feasibility is then demonstrated by incorporation of the biomarker into clinical radiological practice or by qualification for use in clinical trials.

Survey of Current cSVD Biomarker Development with Specific Considerations for Selected Emerging Modalities

CommonlyThe most studied neuroimaging biomarkers of cSVD are lacunes, WMH of presumed vascular origin, perivascular spaces and cerebral microbleeds. With the exception of perivascular spaces, Tthese lesions are typically described reported in routine radiology reports in clinical practice and have been incorporated as secondary imaging endpoints in some clinical trials. For these markers proof of concept, principle, and effectiveness have been established. Even so, longitudinal data on change over time and data on repeatability and reproducibility, so important for planning sample sizes in clinical trials, are relatively scant[15,16].

A recent systematic review highlighted the gaps in knowledge in repeatability and reproducibility of measurements of cSVD lesions, focusing mostly on quantitative

biomarkers including volumes of WMH, lacunes, and brain[17]. The authors systematically searched the literature to identify information on scan-rescan repeatability (which they termed "within center reproducibility") as well as the effects of scanner vendor, field strength, sequence choices, and coil type. They found that the amount of literature on repeatability and reproducibility varied widely by lesion type. The most literature was found on measures of brain volume, probably because brain atrophy is an important biomarker for many neurological diseases in addition to cSVD, such as Alzheimer's disease, and because phantoms are available for measuring variations in geometric distortions across scanners. For WMH, lacunes, perivascular spaces, and microbleeds there was only sparse information on repeatability with relatively speaking the greatest amount of information on WMH measurements cross-sectionally, but no repeatability data on longitudinal measurements.

Figure 2 provides an overview of the validation status of the best established cSVD markers as well as emerging modalities and techniques. Over time the list of neuroimaging biomarkers of cSVD has grown substantially as our knowledge of cSVD pathophysiology[2], and ability to image it, has grown.

Some markers have already received a large amount of attention, notably WMH (assessed visually or computationally), lacunes, and microbleeds (mainly visually with some emerging computational methods). Even so, some aspects of validation are lacking with few large comparisons of different volumetric tools, little longitudinal data, and none are yet adopted as confirmed surrogate outcomes in clinical trials. Nonetheless, they have already been the subject of many reviews[16,17].

Hence, the list of biomarkers discussed in detail here represents the subset that the HARNESS group selected as the next most promising for measuring unique aspects of cSVD pathophysiology, but that have so far received less attention. The list is not exhaustive. Future research will likely add more modalities and lesion types. For example, microinfarcts have

been visualized on MRI by several research groups and may be a frequent but underrecognized consequence of thrombosis or embolism of small arteries[18]. Additionally, future research ~~and~~ may clarify that biomarkers currently on the list are a poor fit for some purposes.

In the following sections, we review the state of imaging biomarker development for ~~these~~ selected emerging modalities, along with considerations for further development and harmonization.

***Structural Imaging:* Perivascular spaces**

Perivascular spaces are rapidly emerging as a novel marker of cSVD and are defined as "fluid-filled spaces that follow the typical course of a vessel as it goes through grey or white matter"[5]. While long considered an innocuous phenomenon of aging, a converging body of proof of principle cross-sectional studies now suggests that a larger burden of perivascular spaces is associated with a higher likelihood of dementia, cognitive impairment, and stroke[19-21] More importantly, these associations are independent from established markers of cSVD. Longitudinal studies of the appearance of perivascular spaces or their enlargement over time are lacking; therefore, the rate at which these spaces change over time is essentially unknown. One study showed that the 5-year incidence of new large perivascular spaces (defined as ≥3 mm diameter) in a general elderly population was 3.1%[21], however this size exceeds the generally accepted current width boundary between perivascular spaces and lacunes[5].

There are few data on the repeatability of measurements of perivascular spaces and reproducibility of measurement across scanners. For one automated method, repeatability was excellent with intra-class correlations of 0.92 for basal ganglia and 0.87 for centrum semiovale[22]. In contrast, intra-rater and inter-rater reliability for visual rating scales have been published by several groups and should be expected to be good to excellent (i.e., with kappa values of 0.5 or higher or intra-class correlation coefficients of 0.6 or higher). Rating on T2-

weighted sequences is favoured because perivascular spaces are well visible, but some studies have used high resolution T1-weighted sequences instead. In one study, ratings on T1-weighted and T2-weighted sequences showed excellent correlation (intraclass correlation >0.80)[23].

The HARNESS working group identified several difficulties in the quantification of perivascular spaces, which have so far hampered comprehensive understanding of their biological meaning. First, perivascular spaces, reflecting the virtual space between blood vessels and pia mater, by themselves are a physiologic finding. It is the enlargement of these spaces that can be visible on MRI that is considered non-physiologic. The question then remains what amount of enlargement should distinguish physiologic from non-physiologic perivascular spaces? Originally, a convenience threshold was chosen, such that any perivascular space visible on brain MRI was considered enlarged. However, increasing field strengths and other advances in imaging now allow much smaller perivascular spaces to become visible on MRI, indicating the need to use a more objective and reproducible threshold independent from imaging parameters.

Second, since perivascular spaces are defined by their intricate relation to brain vessels, they are ubiquitous in all brain regions. Yet, the extent of enlargement is different across brain regions and should be taken into account in their quantification. A working upper width limit of 3 mm is widely used to discriminate perivascular spaces from small lacunes[5], but for example it is well recognized that larger width perivascular spaces are sometimes seen in the substantia innominata. Radio-pathological correlation studies show that MRI can differentiate perivascular spaces from lacunes with good sensitivity and specificity using morphological and signal intensity information[24], but more validation on correlations by region would be welcome. Similarly, the processes underlying their enlargement are thought to differ according to brain region; for example, in cerebral amyloid

angiopathy enlargement of perivascular spaces is seen in the centrum semiovale but not in the basal ganglia.[25,26].

Against this background, it is not surprising that the various efforts to quantify perivascular spaces have differed with respect to definition of enlargement, regions to be scored, and scoring system used[23,27-30]. While work continues to identify the key features of these rating systems with respect to similarities, dissimilarities, strengths, weaknesses, and 'translation' from one rating system to the other, we recommend that investigators use the rating system most relevant to their population, or that they are most comfortable with, while having a core understanding how that specific rating system relates to others available in the literature. Raters should be trained on a standardized dataset with measurement of intra-rater and inter-rater reliability and report these measures in publications; training tools are available on HARNESS website.

Parallel to this development of visual rating, there is now a strong focus on fully-automated quantification of perivascular spaces. These efforts have so far been hampered by similar methodological considerations as outlined above, but the recent introduction of machine learning algorithms in brain imaging holds great promise in overcoming these barriers[22,31]. Just like automated quantification of WMH resulted in dramatic improvement in our understanding of their role in neurodegenerative diseases particularly at the voxel level, automated detection, volumetrics, shape, density and orientation of perivascular spaces could signify a paradigm shift in their position within the pantheon of cSVD markers.

***Structural Imaging:* Atrophy in the context of cSVD**

Atrophy is now a well-established, measurable consequence of cSVD. Both cross-sectional and longitudinal studies show proof of principle that total brain volume is lower in cSVD and decreases more quickly in persons with enlarging WMH. ~~the context of progressive cSVD.~~ The repeatability and reproducibility of brain volume measurements in the

context of cSVD has been reviewed recently[17]. Here, we highlight specific aspects to be considered when implementing atrophy measurements in cSVD studies.

Given the complexity of brain anatomy, measures of brain volume should be obtained from 3D T1-weighted high-resolution isotropic sequences with quantitative computerized methods where possible. To capture chronic, final effects, the image acquisitions should be performed remotely in time (probably 90 days or longer) from the occurrence of acute brain lesions.

At a given time point, volumetric measures reflect the sum of the individual's maximum brain volume growth (estimated by the intracranial cavity volume), the effect of age, and that of multiple potential diseases including cSVD, overt stroke, and neurodegenerative diseases such as Alzheimer's disease. Controlling for differences in head size, e.g. by expressing volumes as a fraction of intracranial volume or including intracranial volume as a covariate, is mandatory in single time point analyses. Although controlling for intracranial volume is not strictly necessary for longitudinal analyses, investigators may still want to analyze it as a proxy for original maximum brain size which reflects premorbid brain health and is associated with general intelligence[32]. In longitudinal analyses, the use of cross-timepoint registration pipelines rather than repeated use of cross-sectional methods may reduce variability in measurement[33,34] but the optimal approach remains to be confirmed.

Methods involving registration to a common template should be used cautiously given that brains with cSVD, often exhibiting large ventricles and white matter atrophy, can register poorly to atlases based on healthy individuals. This is a particularly challenging problem when cSVD is accompanied by larger destructive intracerebral hemorrhages or infarcts. The impact of brain tissue lesions on the different methods to assess brain volume is often unpredictable[35]. In particular, the presence of extensive WMH can lead to erratic behavior of most algorithms,[36,37] and if appropriate they should be masked. Additionally, algorithms may

variably segment fluid-filled cavities within the brain (lacunes and enlarged perivascular spaces) as cerebrospinal fluid, gray matter or white matter, requiring a systematic visual quality control of segmentation results[35,38]. There is consensus that cavities resulting from infarction should be excluded from ~~These fluid-filled cavities should be excluded from~~ brain tissue estimates[5], depending on the question being asked; clearly, they do not represent spaces such as subarachnoid space or ventricles but nor do they represent normal brain tissue. They can be considered as part of the 'total burden of brain injury'[39] in some analyses. Quantitative methods are emerging that can estimate perivascular space volume; when such measurements are made we recommend that perivascular space volume be reported as a separate tissue class and not included in the total brain volume. Given the numerous sources of variation in gray to white contrast in cSVD, differential measures of gray and white matter volumes should be interpreted carefully[40]. The use of other computational volumetric markers, such as ventricle volumes, has not been validated in cSVD. All methods require visual checking and may need manual editing where automated segmentation has failed to identify the correct tissue.

Diffusion imaging metrics

Diffusion imaging provides of the diffusion of water molecules within brain tissue. There are a large variety of techniques to analyze these data. Diffusion-weighted imaging is positive (that is, shows increased signal) in the setting of recent infarction or microinfarction. Scalar measures describe diffusion properties on the voxel level, such as the extent or directionality. Diffusion tensor imaging (DTI) is the most useful model to derive these scalar metrics such as mean diffusivity (MD) or fractional anisotropy (FA). Tractography can be used to visualize fiber connections and analyze diffusion on the tract level. Global tractography in combination with graph theoretical network analysis allows to assess the impact of cSVD on the level of brain networks.

Proof of principle that diffusion imaging metrics can serve as biomarkers of cSVD is well established by multiple studies associating diffusion imaging indices derived from the white matter (WM) or normal-appearing WM (NAWM) with cSVD and cSVD risk factors. Most studies report cross-sectional associations between lower FA or higher MD and cognitive and gait impairments[41,42] Mean diffusivity is readily measured in the whole brain, tissue subregions, regions of interest or tracts and shows the strongest associations with SVD lesion burden[43]. Recent, promising post-processing methods to increase the reliability and ease of extraction of diffusion imaging metrics include histogram-derived diffusion imaging metrics, such as the peak width of the skeletonized MD distribution (PSMD)[44], and connectivity measures including ones based on network theory[45-47]. Lower brain connectivity in strategic network locations, such as long-distance fibers connecting so-called network 'hubs', show promise for prediction of speed and executive functioning[48,49]. This is not an exhaustive list, as there are several other promising diffusion imaging acquisition and analysis methods which show promise for development as biomarkers of cSVD[50,51].

In contrast to the many cross-sectional studies, there are fewer studies evaluating diffusion imaging as a prognostic marker of disease progression.[41] The LADIS study reported an association between NAWM MD at baseline and decline in processing speed,[52] whereas the RUN DMC study found no association between baseline NAWM MD and cognitive decline[53], or risk of dementia over 5 years[54]. Diffusion imaging-derived brain connectivity predicted conversion to dementia after 5 years[55]. Longitudinal studies of diffusion imaging change over time are at this time relatively scarce[56-60] but promising, suggesting that change over time can be detected on diffusion imaging with similar sensitivity as change over time in WMH volume, requiring smaller sample sizes than required to detect atrophy or incident lacunes[61]. Progression over time in diffusion imaging metrics has been associated with increased risk of dementia[58] and gait decline[62].

The tissue correlate of altered diffusion metrics in cSVD is still debated. A recent study suggests that increased extracellular water content is a major contributor[50].

There are few studies on repeatability and reproducibility. The only study in patients with cSVD showed high reproducibility of PSMD in 7 patients with CADASIL scanned on a 1.5T and 3T scanner (intraclass correlation 0.95)[44]. Other studies in healthy controls have shown good repeatability and reproducibility for FA and MD measurements (coefficient of variation ranging from 0.8 to 5.7%)[63-65]. Nonetheless, variation in scanner or scanner upgrades may bias measurements in longitudinal studies[63]; therefore, investigators ideally should avoid scanner upgrades or changing scanners between baseline and follow-up measurements in studies designed to detect small changes over time. Phantoms to estimate reproducibility are in development.[66]

Perfusion and cerebrovascular reactivity

Perfusion and cerebrovascular reactivity (CVR) approaches are highly relevant in cSVD research because reduced tissue perfusion and impaired CVR are hallmark pathological features. These physiological forms of imaging introduce a unique set of challenges for study design, given the large variability in acquisition methods for perfusion and especially CVR which are less well established compared to many structural imaging techniques. To image CVR, the investigator must choose among several experimental methods for stimulating changes in cerebral blood flow (CBF), as well as between several different acquisition types such blood oxygen level dependent (BOLD) or arterial spin labeling (ASL). Because the vascular signal comes from only a proportion of voxel contents (the blood volume fraction in grey matter accounts for 5 to 10% of the tissue volume), and for BOLD-related techniques the changes in hemoglobin oxygenation are relatively small, attention must be paid to ensure sufficient signal to noise ratio to generate images of adequate quality.

Dynamic susceptibility contrast (DSC) and ASL are examples of MRI acquisitions that yield perfusion-weighted images; the former relies on an exogenous gadolinium contrast agent, while the latter uses magnetically labeled arterial blood water that is proximal to the imaging volume to label blood and produces quantitative perfusion maps typically expressed in units of mL/100g tissue/minute.

ASL is a promising modality for repeated measure studies because it does not require administration of an exogenous intravenous contrast agent. A fraction of cSVD articles on perfusion have thus far used ASL[67]; cross-sectional studies, for example, provide proof of principle by showing that a pattern of reduced frontal perfusion was associated with increased WMH volume[68]. Longitudinal studies are less common, however, one 4-year follow-up study reported that global CBF decreases were associated with higher baseline WMH but that baseline CBF was not associated with greater WMH progression.[69] Another longitudinal study found that while lower baseline CBF predicted appearance of new WMH at 18 months, change in CBF was not associated with new WMH[70]. Studies are needed on the association of baseline and longitudinal CBF and the prevalence and incidence of new brain infarcts and microinfarcts. Although white matter and subcortical tissue perfusion estimates are of particular interest in cSVD, these measurements are less robust than in grey matter when using ASL[71] due to the lower CBF and longer arterial transit time.

A validation study of ASL found higher repeatability for pseudo-continuous ASL compared with pulsed ASL or continuous ASL, with a coefficient of variation of 3.5% in gray matter and 8.0% in white matter[72]. There are few reproducibility studies across scanner types. One study found high reproducibility in eight volunteers scanned on two General Electric (GE) 3T scanners[73]. Another study found that sequence parameter differences had a larger effect than hardware or software differences on General Electric, Philips, and Siemens scanners[74]. Phantoms for ASL have been developed but not yet widely adopted[75].

Unlike physiological imaging during a single "baseline" state, CVR involves physiological provocation to measure a vasoactive response, typically by breathing medical air enriched with carbon dioxide gas. Technical and paradigm details and considerations have been recently reviewed[76]. Multi-contrast physiological imaging, combining perfusion and CVR maps in cSVD, is a promising technique[77]. At this time, relatively few CVR studies have focused explicitly on cSVD[78]. However, CVR imaging is being exploited as an imaging endpoint to assess the efficacy of vasodilatory drugs in a dose escalation trial[79]. CVR appears to be a promising prognostic biomarker of cSVD brain changes, for example as revealed by one longitudinal study that found impaired regional CVR was predictive of WMH lesion expansion at one-year follow-up[80]. A four-year longitudinal study showed that age-related decreases in CVR were associated with steeper declines in processing speed and episodic memory but not working memory or reasoning; however, the degree to which enlarging WMH or new infarction ~~progressive cSVD~~ may have ~~caused~~ been associated with these changes was not assessed. The BOLD-response to a visual stimulus has been shown to be a possible biomarker for CAA and could be a more easily implemented, well-tolerated alternative means to measure CVR, but is is limited to the occipital lobe[81-83] and has not been compared directly to CVR measurement based on hypercapnia.

The repeatability of CVR measurements has been investigated in healthy controls but not patients with cSVD. In a study of 15 controls, the coefficient of variation ranged from 7.3% to 42.9% across 16 regions of interest including cortical and subcortical grey matter and white matter[84]. The coefficient of variation was lower when using a paradigm that averaged two three-minute blocks of CO_2 inhalation rather than three one-minute blocks[84].

A consensus group has provided recommendations for ASL imaging protocols[85]; however, long-label and long-delay ASL approaches may prove superior for CBF measurement in the white matter and subcortical gray matter. Multicenter studies using

scanners from different vendors seems justifiable as long as key methods (including choice of pseudo-continuous ASL, readout strategy, labeling duration, and post-labeling delay time) are kept constant. For CVR imaging, there are a greater diversity of methods and the different methods may suit specific patient populations. One published protocol[84] using three-minute CO_2 blocks is being used in a multicenter trial.

Blood-brain barrier integrity

Although proof-of-concept evidence is very limited, proof-of-principle evidence from cross-sectional clinical studies suggests that blood-brain barrier (BBB) dysfunction determined by MR is associated with imaging features of cSVD, and that BBB leakage may contribute to tissue damage, development of cSVD features and long-term adverse outcomes[86,87]. Therefore, BBB permeability is an important target of measurement in studies of pathophysiology and treatment evaluation.

Dynamic contrast-enhanced MRI (DCE-MRI) using a standard dose of a gadolinium-based contrast agent is presently the most promising technique for quantitative imaging of subtle leakage[86], and has been applied in several studies of cSVD and related conditions.[86,88-91] However, while the technique is well-established in other conditions such as brain tumours, particular challenges emerge in cSVD due to the slow rate of leakage. For qualitative assessment, gadolinium-based contrast agent (GBCA) enhancement of cerebrospinal fluid on T2-weighted fluid attenuated inversion recovery (FLAIR) and T1-weighted imaging may provide a practical, though non-specific, alternative[92,93]. Other potential methods are difficult to quantify (e.g. dynamic susceptibility contrast MRI),[94] employ ionising radiation,[95,96] or are at an early stage of development (compartmental ASL modelling[97-99]). Nevertheless, DCE-MRI is not routinely used in cSVD studies due to practical impediments (long scan time, exogenous contrast), lack of widespread expertise, and technical and physiological complexities and confounds[100,101].

There are few studies of BBB permeability change over time in cSVD. A single study of 22 subjects with high WMH burden reported little overlap between regions of high white matter permeability between the first and second scan, but that high permeability was often seen along the border of WMH at either time[102].

Because there is no reliable convenient reference method for quantifying subtle BBB permeability, studies comparing DCE-MRI measurements with other measures of BBB integrity are few and inconclusive[103,104]. The need for a second gadolinium administration is a barrier to conducting studies on repeatability, but one study showed good evidence of repeatability with coefficient of variation of 11.6 % for white matter and 14.4 % for gray matter at 3T[105]. Reproducibility across different MR hardware has not been investigated. Based on theoretical considerations and experimental observations, it is likely that measurements are influenced by MR field strength, scanner stability, spatial resolution, pulse sequence parameters, acquisition time, GBCA type, and pharmacokinetic model[100,101,106,107]. The diversity of acquisition and analysis protocols described (sometimes incompletely) in the literature is, therefore, a key impediment to the interpretation and comparison of data from different studies and centres.

Our recommendation for future studies is to use a three-dimensional, MR acquisition with wide spatial coverage, pre-contrast T1 measurement, a minimum temporal resolution of around one minute and minimum DCE scan time of 15 minutes[108]. A vascular input function should be measured in the venous sinuses and the permeability-surface area product *PS* for tissue regions or, where feasible, individual voxels should be estimated using an appropriate pharmacokinetic model, typically the Patlak model[109]; simulations may be performed to assess accuracy and precision. Results should be interpreted carefully, particularly when comparing data from different research groups or scanners. We identify three priorities for the development of this biomarker: (i) agreement by the wider cSVD and dementia imaging

research community on an open-access, dynamic consensus protocol for DCE-MRI measurements of slow BBB leakage, (ii) acquisition of data on repeatability and reproducibility, and (iii) studies to assess accuracy, including theoretical work, comparison with independent measures of BBB integrity, and validation using MR test objects and histology. Further technical development to increase accuracy and precision, as well as continued development of alternative methods are also encouraged.

Ultra high field MRI

Ultra-high field MRI, in particular 7T MRI, is emerging as a new tool in cSVD research. The higher resolution, different tissue contrasts, and better signal to noise ratios of 7T MRI allow the investigator to probe aspects of cSVD that are difficult to assess at lower field strength. In addition to enhanced sensitivity for cSVD lesions such as microinfarcts and microbleeds and more precise assessment of atrophy[18,110], with 7T MRI it is possible to actually visualize the small vessels[111]. From both perforating arteries and veins features such as vessel density, length, and tortuosity can be resolved.[111,112]. Additionally, different aspects of vascular function, including blood flow, pulsatility of flow in small penetrating arteries (a possible indicator of vascular stiffness), vascular reactivity to vasoactive agents (e.g carbon dioxide) or neuronal stimulation (i.e. functional MRI), can be assessed, making it possible to probe cSVD at the level of the small vessels themselves[111].

Despite the potential for of 7T MRI for cSVD, important steps have to be taken to validate these novel techniques. Of note, EUFIND (the European Ultrahigh-Field Imaging Network in Neurodegenerative Diseases), another JPND initiative, has the goal of harmonizing 7T MRI protocols across more than 20 centres from Europe and the US.

Tools to Facilitate cSVD Biomarker Development and Harmonization

The HARNESS initiative focused on three areas to provide tools for harmonization: MR acquisition, post-processing, and common repositories for training and validation. These tools are made available to the research community at www.harness-neuroimaging.org.

The HARNESS website provides fully specified MR acquisition protocols suitable for research studies that include a focus on cSVD. Given the diversity of manifestations of cSVD and hypotheses that can be tested, there is no single MR acquisition protocol that can quantify all aspects of cSVD and therefore investigators must make choices regarding protocol composition, also accounting for issues of feasibility including acquisition time and cost. Therefore, instead of a single protocol the HARNESS website provides several options that meet these criteria: a) they adhere to STRIVE[5], b) they are suitable for identifying canonical cSVD lesions types--lacunes and WMH of presumed vascular origin, recent small infarcts, microbleeds, atrophy, and DTI changes, c) they have been tested on more than one scanner as part of an established multicenter study and d) the protocol developers are willing to share the protocol freely. There are also links to other websites and useful repositories of information.

Currently, protocols are available from the SVD@target study[84] (ISRCTN10514229) and the Canadian Dementia Imaging Protocol[113], with plans to add the protocol from the U.S. National Institute of Neurological Disorders and Stroke MarkVCID Biomarker Consortium (https://markvcid.partners.org/) once it has been fully specified and tested. Sequence parameters with exam cards are provided for 3T for most of the major vendors including General Electric, Phillips, and Siemens. The protocols are suitable for prospective research studies with quantitative imaging biomarkers but probably exceed most clinical stroke protocols in terms of acquisition time, spatial resolution, and inclusion of DTI. They have been implemented successfully in multicenter studies at research sites, but nonetheless may not be feasible for multicenter studies performed at predominantly clinical scan sites where the intent is to leverage clinical imaging without a focus on quantitative biomarkers.

Reducing imaging variability may be enhanced by following consensus recommendations[17] to perform automated quality checks for acquisition parameters and monitoring of images for artefacts, correction for gradient nonlinearities, a well-defined method for subject's positioning in the scanner, and a clear strategy for hardware replacement when needed.

The HARNESS software database provides a searchable source for information on downloadable software tools for processing MR data for cSVD quantitative biomarkers, such as for segmenting WMH. There are many existing software libraries for neuroimaging analysis, but only HARNESS focuses exclusively on cSVD. Site users can search for software by image modality, measurement type, key words, availability (i.e. by download or by request to the developer), or operating system. Software developers control their own entries via password-protected accounts, and must make their software available according to their own terms by providing a link or through contacting the developer. We are actively recruiting developers with tools to sell or share. Developers may access the site for information on how to create accounts.

To aid visual review for cSVD lesions according to STRIVE, the HARNESS site makes downloadable electronic documents available including validated visual rating scale scores and instructions, case report forms, and training slides.

Training readers and software algorithms requires access to independent MR datasets for measurements. The HARNESS site includes a web-based repository with completely de-identified 3T MR data showing lacunes, WMH, microbleeds, and cortical superficial siderosis from patients with TIA, minor ischemic stroke, and cerebral amyloid angiopathy, with consensus "gold standard" measurements for comparison. This repository will be useful for independently confirming reliability of measurements within and across research groups, and for derivation and validation of computerized algorithms for quantitative measurement

(e.g. for segmenting WMH to determine location and overall volume) as well for comparing WMH algorithms against an independent standard.

SUMMARY AND CONCLUSIONS

The HARNESS initiative was a multidisciplinary consensus process with input from a large number of neuroimaging researchers investigating cSVD. Our group developed a framework for neuroimaging biomarker development closely aligned with those proposed in other areas of imaging research. The HARNESS website (www.harness-neuroimaging.org) was created to facilitate harmonized neuroimaging methods for cSVD research. The site includes cSVD-appropriate MR acquisition protocols aligned with STRIVE, a searchable database of softwares for analyzing brains with cSVD, visual rating scales and case report forms, and a repository of 100 deidentified scans demonstrating different cSVD lesion types. These tools and resources are made available to the research community via the site and can be easily updated by contributors.

In this rapidly evolving field, we found that the degree of biomarker validation—technical, biological and clinical, and feasibility—varied by cSVD lesion and measurement type. In general, visually diagnosed cSVD lesions such as lacunes, WMH, and microbleeds have the greatest amount of clinical validation including as prognostic markers and data are available on incidence andchange over time, and are already being used in multicenter studies and reported in routine clinical practice. Even so, none of these markers has yet been qualified for use in clinical trials by regulatory agencies, and more work is needed to standardize and compare current volumetric tools. Other markers are at a less advanced stage of biomarker development. Atrophy has been extensively studied but almost always in the context of Alzheimer's disease and not cSVD. Among the emerging cSVD markers there are relatively more data on diffusion imaging and perivascular space imaging, but more

longitudinal data and multicenter data on reproducibility are needed. Measurements of brain perfusion, vascular reactivity, and blood-brain barrier integrity are promising but are at an even earlier stage of development. For these cSVD manifestations innovation to overcome technical and feasibility barriers, rather than harmonizing to a best protocol, is the most important next step in development.

We found that technical validation often lagged clinical validation. However, estimates of repeatability and reproducibility are critically important to estimate minimum detectable differences over time and variability in measurement in multicenter studies, essential for sample size calculations for multicenter longitudinal trials. This lag in technical validation likely reflects the difficulty in obtaining funding for technical studies compared to clinical studies, the burden on research subjects to undergo multiple scans, and the general lack of non-human phantoms for studies of reproducibility. In contrast to volumetric imaging and functional MRI, phantoms for other measurements are less well developed. One research group has developed a phantom for iron deposits that mimic mineral deposits and microbleeds, not currently available for purchase[114]; otherwise, we are not aware of any other phantoms that recreate specific aspects of cSVD. Technical validation for neuroimaging biomarkers of cSVD would be enhanced by creating funding opportunities specifically for this purpose.

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KEY WORDS: Cerebrovascular disease, stroke, dementia, magnetic resonance imaging

Figure 1. Imaging Biomarker Development Framework for Cerebral Small Vessel Disease

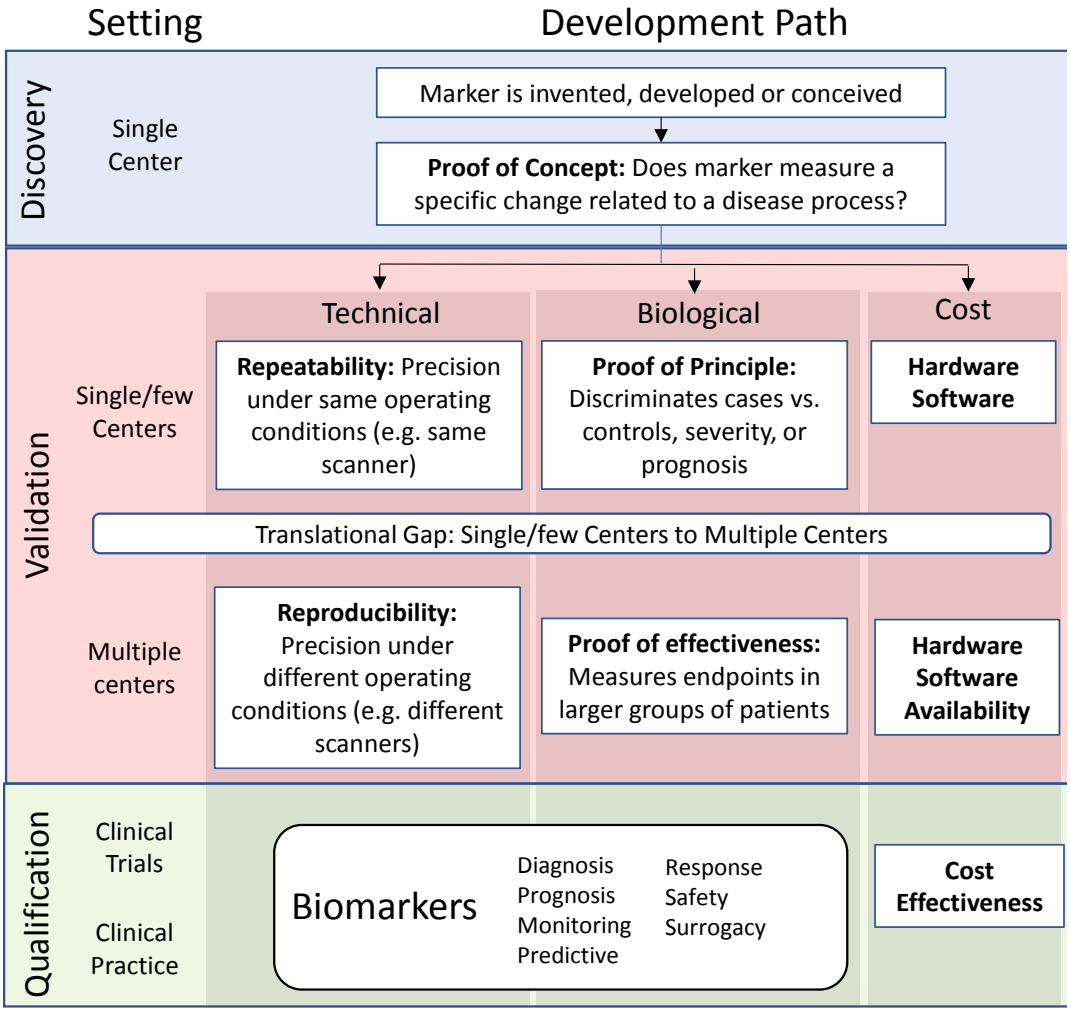

Figure 2. Schematic overview of neuroimaging biomarker development status for cerebral small vessel disease

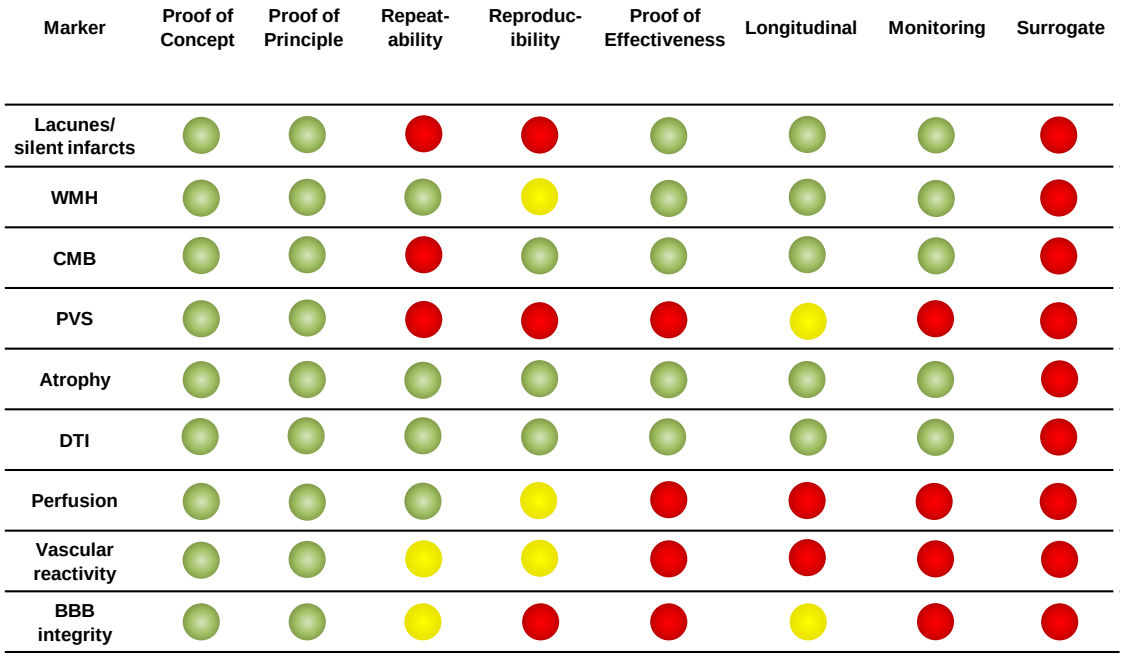

Figure 2 Legend: Green light indicates validation data from two or more studies from independent research groups; Yellow light indicates support from a single study or conflicting evidence from multiple studies; Red light indicates there is currently insufficient evidence. WMH, white matter hyperintensities of presumed vascular origin; CMB, cerebral microbleeds; PVS, perivascular spaces; DTI, diffusion tensor imaging; BBB, blood-brain barrier. *Proof of concept*: evidence that the marker measures a specific change or process related to cerebral small vessel disease. *Proof of principle/ Mechanism*: evidence that the marker differs between patients with and without cerebral small vessel disease. *Proof of effectiveness*: evidence from larger scale multiple center studies that the marker differs between patients with and without cerebral small vessel disease. *Repeatability:* precision of repeated measurements under the same conditions using the same scanner. *Reproducibility:* replicate measurements on the same or similar objects (e.g. a phantom or human volunteers) in different locations using different scanners. *Longitudinal:* rate of change over time has

been defined. *Monitoring:* evidence that longitudinal change in the marker is associated with progression of cerebral small vessel disease. *Surrogate*: evidence that change in the marker is strongly associated with clinical outcomes in cerebral small vessel disease, such that changes in the marker could be considered a substitute for a clinical endpoint.

General comment: Three additional working group members contributed to the manuscript revisions, whom we would like to add as coauthors: Dr. Walter Backes, Dr. Michael Ingrisch, and Dr. Stefan Ropele.

The reviewers have now commented on your paper. Both really support this publication, but reviewer #2 has offered some suggestions for improvement.

Response: We appreciate the reviewer's interest in our work.

Reviewer #1: This is an excellent overview and model, with great summary graphics to demonstrate current state of the art for MRI biomarker development to measure vascular contributions to NDG diseases. I think this is a useful contribution to the planned JPND special section. Well written, authoritative and clear.

Response: Thank you.

Reviewer #2

This is an important and well-written study with a direct end-result, a website, that directly will benefit further research on the topic. Thanks for a very well written study!

Response: Thank you.

1. "The most studied neuroimaging biomarkers of cSVD are lacunes, WMH of presumed vascular origin, perivascular spaces and cerebral microbleeds " Sure, these markers are certainly the most studied, but perivascular spaces has not been studied to the same degree as the other mentioned markers. I suggest not including "most studied" as it is not based on evidence, and may be inaccurate. "Commonly studied" may be another way to accurately phrase this.

Response: We have revised the sentence as follows (page 8): "Commonly studied neuroimaging biomarkers of cSVD are lacunes, WMH of presumed vascular origin, and cerebral microbleeds. These lesions are typically reported in routine radiology clinical practice and…."

2. In the atrophy section perivascular spaces are briefly touched upon with regards to spaces not to be included in the final brain segmentation volume. Is there however software that measures the total volume of perivascular spaces? Please include a brief sentence on this.

Response: Software to calculate perivascular spaces are just beginning to be developed. An example of one such method is provided in the section on perivascular spaces (reference 31). However, perivascular space volume measurement is currently not implemented in the most commonly used packages for brain segmentation, such as FSL or Freesurfer. We have made a revision as follows (page 14): "There is consensus that cavities resulting from infarction should

be excluded from brain tissue estimates[5] depending on the question being asked; clearly, they do not represent spaces such as subarachnoid space or ventricles but nor do they represent normal brain tissue. They can be considered as part of the 'total burden of brain injury'[38] in some analyses. Quantitative methods are emerging that can estimate perivascular space volume[30]; when such measurements are made we recommend that perivascular space volume be reported as a separate tissue class and not included in the total brain volume."

3. "progressive cSVD." Define progressive cSVD

Response: We have revised the manuscript in two places to be more specific. On page 11 we now write: "Both cross-sectional and longitudinal studies show proof of principle that total brain volume is lower in cSVD and decreases more quickly in persons with enlarging WMH." On page 18 we now write: "A four-year longitudinal study showed that age-related decreases in CVR were associated with steeper declines in processing speed and episodic memory but not working memory or reasoning; however, the degree to which enlarging WMH or new infarction may have been associated with these changes was not assessed."

4. Diffusion imaging metrics is discussed but acute microinfarcts are not mentioned. This may be worthwhile including.

Response: We have added this sentence acknowledging the important role of diffusion weighted imaging in identifying acute infarction, including microinfarction (page 14): "Diffusion-weighted imaging is positive (that is, shows increased signal) in the setting of recent infarction or microinfarction."

Additionally, we have revised the section on the Survey of Current SVD Biomarker Development to cite a recent review of microinfarcts published in Lancet Neurology (page 9, reference 18): "Future research will likely add more modalities and lesion types. For example, microinfarcts have been visualized on MRI by several research groups and may be a frequent but underrecognized consequence of thrombosis or embolism of small arteries."

5. Interesting section on perfusion. It would be great if one sentence or two could be included on cortical microinfarcts and their association with brain perfusion.

Response: Several lines of evidence point to an association between hypoperfusion and cortical microinfarcts including experimental animal studies, and human pathology studies that identify a higher frequency of microinfarction at the borderzones of cerebral arterial territories. However, to our knowledge there are not yet human *in vivo* data that directly associate hypoperfusion with the presence of microinfarction. Establishing the role of hypoperfusion in causing microinfarcts is listed as a future research direction in a recent review of microinfarcts published in Lancet Neurology (reference 18). We have revised the perfusion section to cite the need for longitudinal studies on perfusion and microinfarcts (page 19): "Studies are needed on the association of baseline and longitudinal CBF and the prevalence and incidence of new brain infarcts and microinfarcts."

6. "attention must be paid to ensure sufficient signal to noise to generate images of adequate quality" Add ratio after "signal to noise"

Response: We made the suggested revision (page 16).

7. Write out GBCA first time it is used since this is not an imaging journal per se.

Response: We have spelled it out: “gadolinium-based contrast agent” (page 19).

8. General: All the subtitles under results include imaging techniques, however perivascular spaces stand out in that they are a marker of cSVD. I think the paper would benefit from PVS being part of the atrophy section. Or maybe under a separate title named structural imaging.

Response: To better harmonize the sections we have moved the perivascular spaces section to follow atrophy and renamed these sections “Structural imaging: brain atrophy” and “Structural imaging: perivascular spaces”.

Figure 2

Figure 1