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MarkVCID Cerebral small vessel consortium: I. Enrollment, clinical, fluid protocols

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Conflicts of interest

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EXECUTIVE SUMMARY

The concept of vascular contributions to cognitive impairment and dementia (VCID) derives from over two decades of research indicating that 1) most older individuals with cognitive impairment have post-mortem evidence of multiple contributing pathologies and 2) along with the preeminent role of Alzheimer's pathology, cerebrovascular disease accounts for a substantial proportion of this contribution. Contributing cerebrovascular processes include both overt strokes caused by etiologies such as large vessel occlusion, cardioembolism, and embolic infarcts of unknown source, and frequently asymptomatic brain injuries caused by diseases of the small cerebral vessels. Cerebral small vessel diseases such as arteriolosclerosis and cerebral amyloid angiopathy, when present at moderate or greater pathologic severity, are independently associated with worse cognitive performance and greater likelihood of dementia, particularly in combination with Alzheimer's and other neurodegenerative pathologies. Based on this evidence, the US National Alzheimer's Project Act explicitly authorized accelerated research in vascular and mixed dementia along with frontotemporal and Lewy body dementia and Alzheimer's disease itself.

Biomarker development has been consistently identified as a key step towards translating scientific advances in VCID into effective prevention and treatment strategies. Validated biomarkers can serve a range of purposes in trials of candidate interventions, including 1) identifying individuals at increased VCID risk, 2) diagnosing the presence of cerebral small vessel disease or specific small vessel pathologies, 3) stratifying study participants according to their prognosis for VCID progression or treatment response, 4) demonstrating an intervention's target engagement or

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pharmacodynamic mechanism of action, and 5) monitoring disease progression during treatment. Effective biomarkers allow academic and industry investigators to advance promising interventions at early stages of development and discard interventions with low success likelihood.

The MarkVCID consortium was formed in 2016 with the goal of developing and validating fluid- and imaging-based biomarkers for the cerebral small vessel diseases associated with VCID. MarkVCID consists of seven project sites and a central coordinating center, working with the National Institute of Neurologic Diseases and Stroke and National Institute on Aging under cooperative agreements. Through an internal selection process, MarkVCID has identified a panel of 11 candidate biomarker “kits” (consisting of the biomarker measure and the clinical and cognitive data used to validate it) and established a range of harmonized procedures and protocols for participant enrollment, clinical and cognitive evaluation, collection and handling of fluid samples, acquisition of neuroimaging studies, and biomarker validation. The overarching goal of these protocols is to generate rigorous validating data that could be used by investigators throughout the research community in selecting and applying biomarkers to multi-site VCID trials.

Key features of MarkVCID participant enrollment, clinical/cognitive testing, and fluid biomarker procedures are summarized here, with full details in the following text, tables, and supplemental material, and a description of the MarkVCID imaging biomarker procedures in a companion paper, “MarkVCID Cerebral small vessel consortium: II. Neuroimaging protocols.” The procedures described here address a range of challenges in MarkVCID’s design, notably: 1) Acquiring all data under informed consent and enrollment procedures that allow unlimited sharing and open-ended analyses without compromising participant privacy rights; 2) Acquiring the data in a sufficiently wide range of study participants to allow assessment of candidate biomarkers across the various patient groups who might ultimately be targeted in VCID clinical trials; 3) Defining a common dataset of clinical and cognitive elements that contains all the key outcome markers and covariates for VCID studies and is realistically obtainable during a practical study visit; 4) Instituting best fluid-handling practices for minimizing avoidable sources of variability; and 5) Establishing rigorous procedures for testing the reliability of candidate fluid-based biomarkers across replicates, assay runs, sites, and time intervals (collectively defined as the biomarker’s instrumental validity).

Keywords

Best practices; biomarker; biospecimen; clinical and cognitive evaluation; clinical and cognitive outcome markers; collection and handling of fluid samples; enrollment; harmonized procedures and protocols; small vessel disease; validation; vascular contributions to cognitive impairment and dementia

1. BACKGROUND

Among the spectrum of pathologies largely responsible for age-related cognitive impairment including Alzheimer’s Disease (AD), AD-related dementias, and vascular contributions to cognitive impairment and dementia (VCID) [1], the contribution of VCID is quite substantial. Both cerebral large vessel pathologies (such as atherosclerosis and macroscopic infarcts) and small vessel disease (SVD) pathologies (such as arteriolosclerosis, cerebral

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amyloid angiopathy, and microscopic infarcts) are highly prevalent in large clinical-pathological studies and independently associated with cognitive performance and decline [2]. In recognition of the public health importance of identifying interventions to slow progression of SVD-related VCID, the US National Institutes of Health (NIH) funded a consortium of academic centers to identify and validate VCID biomarkers. Designated MarkVCID, the consortium's goal is to "evaluate and develop the most promising biomarker candidates…to the point of being ready for large scale multi-site clinical validation studies…of small vessel VCID biomarkers for phase II and phase III clinical trials" [3].

To achieve its mission, MarkVCID selected a panel of specific fluid- and neuroimaging-based candidate biomarker protocols (or "biomarker kits") for validation. The consortium also developed standardized protocols to be applied across MarkVCID study sites guiding participant enrollment, clinical and cognitive testing, collection and handling of fluid samples, and acquisition of neuroimaging data. Biomarker kit testing is designed to encompass both instrumental validation (reliability across users, sites, and time points) and biological validation (association with clinically meaningful aspects of VCID). The vision of MarkVCID is that once a biomarker kit (consisting of the biomarker measure and the clinical and cognitive data used to validate it) is validated for multi-site use, it could be considered ready for multi-site trial applications such as determining SVD-VCID susceptibility or risk, diagnosing SVD presence or subtype, assessing prognosis, monitoring progression, or demonstrating target engagement or mechanism of action for candidate interventions, consistent with FDA guidelines on the use of clinical biomarkers [4].

Here we describe the MarkVCID approach for participant enrollment, clinical and cognitive testing, and sample collection and instrumental validation for the fluid-based biomarker kits (Supplemental Figure A). Enrolling participants with broad consent for multi-site sharing and with thorough characterization of clinical characteristics, vascular history and risk factors, and cognitive performance are fundamental prerequisites for biomarker validation. Markers in blood and cerebrospinal fluid (CSF) have been suggested as informative indicators of pathophysiologic pathways implicated in SVD-VCID such as inflammation, blood-brain barrier breakdown, and endothelial dysfunction [5–7] and provide potentially complementary information to neuroimaging modalities such as MRI. The MarkVCID protocols for multi-site acquisition and instrumental validation of neuroimaging data are reported in a companion paper [8].

2. METHODS

MarkVCID is comprised of seven project sites (Johns Hopkins University School of Medicine [JHU]; Rush Medical Center/Illinois Institute of Technology [Rush/IIT]; Universities of California San Francisco, Davis, and Los Angeles [UC]; University of Kentucky [UKy]; University of New Mexico Health Sciences Center [UNM]; University of Southern California [USC]; and the Cohorts for Heart and Aging Research in Genomic Epidemiology [CHARGE] consortium) and a central coordinating center (Massachusetts General Hospital [MGH]) working with the National Institute of Neurologic Diseases and Stroke (NINDS) and National Institute on Aging (NIA) under cooperative agreements. Consortium decision-making is performed by the MarkVCID Steering Committee,

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comprised of the contact principal investigator from each of the seven project sites, principal investigator and leads of the Coordinating Center (CC) Administrative and Data Cores, and NIH leadership (CC and NIH participants are nonvoting). A series of MarkVCID subcommittees, comprised of representatives with specialized expertise from the project sites and CC, provide recommendations to the Steering Committee for review and approval. Further input to MarkVCID decisions is provided by an External Advisory Committee selected by NIH and interested non-governmental organizations.

Full lists of the membership of MarkVCID committees and subcommittees are in Supplemental Table 1. Methods reported here for MarkVCID participant enrollment were devised by the Protocol and Operations Standardization Subcommittee, consisting of investigators and administrative personnel with expertise in multicenter patient-based research studies particularly in the areas of stroke, cognitive impairment, and biomarkers. Elements of the MarkVCID clinical evaluation, cognitive testing, and structured instruments were selected by the Clinical and Physiologic Data and Cognitive Assessments Subcommittee, consisting of investigators with cognitive research expertise across areas of neurology, geriatric psychiatry, neuropsychology, and biostatistics. Methods for collection and handling of fluid samples and instrumental validation of fluid sample biomarkers were devised by the Fluid-Based Biomarkers Subcommittee, composed of investigators performing fluid biomarker research. All procedures shown below were adopted by consensus of the proposing subcommittees and the full Steering Committee. Prospective enrollment of participants and acquisition of blood samples occur at six MarkVCID sites: JHU, UC, UKy, UNM, USC, and University of Texas Health Science Center San Antonio (UTHSCA, operating as part of CHARGE). Acquisition of CSF samples occur at JHU, UTHSCA, UKy, UNM, and UC. Rush/IIT, University of California Davis, and other CHARGE sites additionally contribute data and fluid samples from previously enrolled research study participants.

3. RESULTS

3.1 Participant Enrollment

The MarkVCID consortium was designed to validate candidate biomarkers for cerebral small vessel diseases across multiple sites and multiple types of participant cohorts ranging across cognitive statuses (normal, mild cognitive impairment, and dementia), vascular risk factor exposures, race/ethnic groups, and recruitment sources (clinic, community, or population-based). The inclusion and exclusion criteria for each subject cohort are summarized in Supplemental Table 2.

The principles guiding subject enrollment were to incorporate informed consent procedures that provide unrestricted access to data and samples for research analysis within and outside MarkVCID without compromising participant privacy or compliance with Health Insurance Portability and Accountability Act protection. These goals were implemented through use of consensus informed consent language incorporated in study documents and informed consent agreements at each enrolling site with review and approval by the site’s governing institutional review board (IRB).

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The MarkVCID consensus language is summarized in Table 1 and shown in complete form online at https://markvcid.partners.org/consortium-protocols-resources (all MarkVCID web addresses accessible after site registration at markvcid.org). The consent language includes statements indicating use of clinical data, image acquisitions, and tissue biospecimens and no limitations on the research for which they could be shared [9–14]. Sharing permission applies to prospectively collected clinical and cognitive data, brain and retinal imaging data, and collected biosamples. Language governing sharing of genetic data and generation of cell lines was based on template language from the Partners Human Research Committee including dbGAP and Genetic Information Nondiscrimination Act (GINA) language [10, 11, 13, 15–19]. The informed consent allows indefinite use of data and clinical samples, that upon written request data would be purged and specimens destroyed, and that research data and specimens already shared could not be purged. The language further indicates that samples could be shared with commercial entities in the development of for-profit discoveries for which participants would not be further reimbursed [20]. The above human subject protection and informed consent language also formed the basis for the MarkVCID Research Agreements that serve as the contract and data and biospecimen sharing agreement. Sites not engaged in prospective enrollment and contributing data from existing research studies (Rush/IIT, CHARGE) signed the Research Agreement and as necessary, addenda outlining specific study requirements.

All participating site protocols, including the MarkVCID template consent language, were approved by site IRBs. Procedures for determining capacity of cognitively impaired study participants and establishing the identification of a legally authorized representative followed local site and state regulations [21]. Informed consent language stipulates that data collection is primarily research-focused and would generally not be shared with participants. MarkVCID policy is that clinical data, conventional MRI scanning, or Clinical Laboratory Improvement Amendments-certified lab results with immediate clinical importance are shared with participants and treating clinicians by the study site.

MarkVCID data is de-identified before being sent to the central data management system. All analyses are performed using de-identified data. Data shared outside of MarkVCID is fully anonymized before release

3.2 Clinical Evaluation, Cognitive Testing, and Structured Instruments

The measures for the MarkVCID minimum clinical dataset were selected to balance coverage of critical domains relevant to VCID against burden on participants and study staff. Another consideration was maximizing overlap with study protocols already in use at the project sites to facilitate their incorporation and cross-site harmonization. The NIA Alzheimer's Disease Center program Uniform Data Set Version 3 (UDS3) [22, 23], in current use at multiple MarkVCID sites and across academic sites outside the consortium, was accordingly selected as the starting point for determination of required elements for MarkVCID clinical data collection, with revisions and additions to meet the specific goals of MarkVCID and for clarity or brevity.

The selected data elements and measures are summarized in Table 2 and are available in complete form at https://markvcid.partners.org/consortium-protocols-resources. Based on

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the degree of subject impairment and implementation at the prospectively enrolling sites, the full clinical data protocol takes approximately two to three hours to complete—60 to 90 minutes for cognitive testing, the remainder for clinical information, physical and neurologic examination, and clinical scales—and is well tolerated by participants. For personal and medical history, the key domains include demographics, vision, hearing and English fluency (for interpretation of neuropsychological tests), history of cerebrovascular disease and other neurological illnesses, history of vascular risk factors, psychiatric and substance abuse history, and family history of cerebrovascular disease and dementia including autosomal dominant conditions. In this area, we include most of the UDS3 demographic elements including level of education and add primary occupation as a correlate of socioeconomic status [24]. Medical history evaluation again uses most of the UDS3 data elements, with less detailed psychiatric history and more detailed history of stroke nature and timing, cardiovascular disease, and pseudobulbar affect. Medication history is simplified to focus on hypertension treatment and family history is reorganized to provide more detail on family history of cerebrovascular disease as well as cognitive decline.

For physical and neurological examination, key elements include basic anthropomorphic measures, blood pressure, and elements of the neurological examination. UDS3 data elements are incorporated with greater focus on stroke-related findings such as lateralized weakness, deep tendon reflexes, visual fields, and somatosensory loss. Because of the sensitivity of balance and gait to cerebrovascular disease [25], we add a quantitative physical measure, the Short Physical Performance Battery [26], which incorporates balance, gait speed, and chair stand.

For cognitive assessments and other rating instruments, the relevant elements include functional assessment tools, behavioral assessments, and neuropsychological testing, particularly measures of executive functioning, processing speed, and memory. We include the Clinical Dementia Rating Scale (CDR, conducted with a participant and informant) [27], Geriatric Depression Scale [28], and most of the UDS3 neuropsychological battery [29] except the Benton Visual Retention Test, which was felt not to offer sufficient information relative to the administration time. Also to reduce testing time, category fluency is limited to animals and letter fluency to F (English version) or P (Spanish version) words (tallied during administration of the Montreal Cognitive Assessment (MoCA)). Domains covered by this battery are summarized in Table 2. To better characterize memory encoding and retrieval, we added a list learning task, allowing sites to select their own list learning measure and record test name, number of items, immediate and longest delayed recall (with duration of delay), recognition hits, and false positives.

In addition to the detailed MRI measures obtained as part of MarkVCID imaging protocols (described in companion paper), we add an ordinal rating of white matter hyperintensities, the Fazekas score [30], with separate ratings of periventricular and deep white matter. For physiologic and genetic measures, sites obtain hemoglobin A1c, cholesterol, high- and low-density lipoprotein cholesterol, total cholesterol, triglycerides, and creatinine and are optionally requested to submit values for C-reactive protein, fasting blood sugar, serum homocysteine, and *APOE* genotype when available, and indicate whether genome-wide association study analysis has been performed.

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To promote uniform implementation of clinical measures across sites, the CC created guidelines available to sites as supplemental instruction manuals or in-line text within the data collection forms (https://markvcid.partners.org/consortium-protocols-resources). Study site staff are required to complete formalized training activities prior to the implementation of protocols with study participants: a prerecorded webinar or training video highlighting key administration, collection and scoring principles for each measure, followed by an online certification test incorporating sample scoring. For cognitive assessments and other rating instruments, clinical training modules include detailed instruction in the administration and scoring of the Fazekas scale, Short Physical Performance Battery, UDS3 neuropsychological battery, and MoCA. Clinician CDR training is also required and available as a public certification program [31]. To maximize inter-rater reliability and to serve as an additional quality control step, each certified rater is asked to send their first three UDS3 neuropsychological battery administrations to the CC for scoring review and feedback by a staff neuropsychologist. Additional training modules in data management and clinical data collection and entry are also available for site personnel.

3.3 Collection and Handling of Fluid Samples

MarkVCID elected to collect and store serum and EDTA-plasma samples for their wide adaptability for biomarker analysis, ease of collection, and stability during standard −80°C storage. Platelet-poor plasma and CSF were later added to accommodate specific biomarker kits. Although none of the fluid-based biomarker kits entail genetic analysis, the consortium elected to store the packed cells remaining after plasma collection to allow future DNA extraction without requiring additional blood draws.

MarkVCID fluid-based protocols were developed to minimize inter-site and inter-batch variability by applying standardized best practices for handling of samples and biomarker assays. A full listing of the MarkVCID Fluid-Sample Best Practice Guidelines is available at https://markvcid.partners.org/consortium-protocols-resources. To limit potential sources of error such as diurnal variation in inflammatory markers [32, 33] or effects of food intake on plasma proteins [34], blood sample collection is recommended and CSF collection mandated for the morning (8–11am locally) while fasting. Samples are to be processed rapidly with needle-to-freezer time under two hours. Other standardized procedures are time between collection and processing, centrifuge speed, spin times, storage vial types, vial sizes, and aliquot sizes (Supplemental Table 3). We selected O-ring cryotubes, an aliquot size of 250μl to minimize waste of samples and freeze-thaw cycles, and 500μl vials to minimize excessive empty vial space and sample lyophilization during prolonged storage. For CSF collection, standard lumbar puncture procedures are used with additional specification of a 25g needle for deep administration of local anesthesia, no extension tubing, recommended use of an atraumatic spinal needle such as the Sprotte 24g atraumatic spinal needle, and avoidance of sample contact with polystyrene. MarkVCID recommends CSF collection under gravity. CSF samples are spun to remove red blood cells and stored in 250μl aliquots (Supplemental Table 3).

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3.4 Instrumental Validation for Fluid-based Biomarkers

Instrumental validation consists of assessment of measurement reliability determined by a biomarker's repeatability (variability of multiple measures under identical conditions) and reproducibility (variability of multiple measures under differing conditions) [35]. For the purposes of MarkVCID fluid-based biomarkers, instrumental validation was operationally defined as: 1) intra-plate and inter-plate repeatability (differences between replicate assays of a single sample performed on a single or multiple assay plates), 2) inter-site reproducibility (differences between a single sample assayed by multiple sites using standardized materials and methods), and 3) test-retest repeatability (differences between multiple samples acquired from the same individual on different days separated by a short time interval, assayed on a single assay plate).

MarkVCID developed common approaches to assessing these instrumental parameters across all fluid-based biomarker kits (Table 3). The fluid-based kits selected by MarkVCID for full validation consist of an Endothelial Signaling (ES) Kit performed on plasma, an Endothelial Inflammation (EI) Kit performed on platelet-poor plasma, a Neurofilament Light Chain (NfL) Kit performed on plasma, and a Cerebrospinal Placental Growth Factor (PlGF) Kit performed on CSF. Detailed descriptions of the selection, sample processing, assay methods, and analytical performance for each MarkVCID fluid-based kit will be published separately.

Intra-plate and inter-plate repeatability will be performed at each participating site using a minimum of eight samples from either control or disease subjects selected to reflect the full range of the measurements. Each sample will be assayed in duplicate or triplicate on three plates on different days and the coefficients of variation (CV) calculated.

Inter-site reproducibility will be determined using aliquots from the same samples sent to all participating sites. 40 plasma samples (ES, NfL), 20 platelet-poor plasma samples (EI), and 20 CSF samples (PlGF) will be selected from across the participating sites, stratified to represent a full range of SVD severities measured by Fazekas Scale score of white matter hyperintensities [30]. Each sample will be assayed in duplicate or triplicate on single plates at each site. Cross-site variation in the distribution of obtained values will be visualized by Bland-Altman plot [36] and the reliability of these measurements estimated using intraclass correlation coefficients (ICC). Analysis of mean levels for individual analytes will be compared to identify systematic bias between sites.

Test-retest repeatability will be determined by obtaining repeat plasma (ES, NfL) and platelet-poor plasma (EI) samples from 10 individuals per participating site returning for three serial blood draws at least five days apart within a 30-day period. Recruitment of individuals for return visits will be stratified so that no more than five of the 10 will be control subjects. Samples from the three timepoints will be assayed on a single plate at each site, the analytical, intra-individual, and inter-individual variability estimated, and ICC calculated across all repeat samples. Because of the high participant burden of serial lumbar punctures, test-retest repeatability will not be assessed for the PlGF kit.

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4. DISCUSSION

The MarkVCID consortium was formed to develop and validate fluid- and imaging-based biomarkers for the SVDs associated with VCID. We describe here MarkVCID's approach to participant enrollment, collection of clinical and cognitive data, handling of fluid samples, and instrumental validation of fluid-based biomarkers. These methods serve as the basis for obtaining data needed to validate the biological properties of the MarkVCID candidate biomarker kits and ultimately for applying the validated kits to multi-site studies or interventional trials.

The core mission of validating biomarkers for multi-site use requires the ability to share data and samples broadly across sites within and outside MarkVCID. This requirement was reflected in the MarkVCID template consent language, which was approved by IRBs at all enrolling sites. The clinical and cognitive elements selected for MarkVCID were aimed at providing sufficient range to allow validation of the various neuroimaging and fluid measures selected by the consortium. These selected elements had to be generic enough to accommodate a range of uses while short enough to be tolerated in different settings and to accommodate additional local or study-specific elements. The MarkVCID clinical and cognitive protocols are intentionally similar to and easily harmonized with the UDS3 protocol, while adding elements of particular relevance to VCID. They also overlap with elements collected in the MarkVCID sites (CHARGE consortium, Rush Medical Center) that have already collected data as part of prior studies and with other national datasets such as the Alzheimer's Disease Genetics Initiative (ADNI) [37] and the Late Onset Alzheimer's Disease Genetics (LOAD) Study [38]. We note that the cognitive test battery was chosen to focus on assessing memory along with processing speed, executive function, and language domains commonly associated with VCID. While these choices were made based on the VCID literature and preliminary data supporting the candidate kits, they are not meant to imply that other cognitive domains are unaffected in VCID.

The MarkVCID fluid handling and processing protocols were designed to address the well-established role of preanalytical factors on variability in fluid biomarker studies [39]. Previous efforts in the AD field have focused on identifying and minimizing these sources of variability via universal preanalytical protocols for blood and CSF to allow direct comparisons between centers and studies [39–42]. The current protocol was based on the Biospecimen Best Practice Guidelines for the Alzheimer's Disease Centers [43]. While this protocol was developed for processing of samples for amyloid and tau analyses, the underlying principles such as documentation of participant physiological factors, standardized time of day for sample collection, rapid processing, use of low binding tubes, and documentation of preanalytical parameters apply to most biomarker research focused on low abundance analytes. Training of participating sites and regular check-in calls with study coordinators are implemented to ensure adherence to protocol, while documentation of deviations allow investigators to avoid samples with incompatible preanalytical variables. Another core MarkVCID principle for fluid-based biomarker validation is to perform assays independently at multiple study sites, avoiding the possibility that only a single lead site can successfully perform an assay. The MarkVCID fluid-based instrumental validation plan requires inter-site comparison of aliquots from the same sample and test-retest stability of

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samples collected repeatedly from individual subjects over a 2–4-week time period, with the goals of harmonizing assay performance across sites and establishing the degree of variability within and between plates, between sites, and between days within a subject. Test-retest analysis will determine whether candidate biomarkers show low short-term fluctuations, a key property for use as measures of long-term, chronically progressive SVD pathologies.

An additional challenge to longitudinal studies like MarkVCID is disruption to patient contact such as that caused by the 2020 COVID-19 outbreak. As acquisition of fluid and imaging data necessitates in-person visits, the Steering Committee elected to delay baseline or longitudinal follow-up visits until permitted by local hospital guidelines rather than attempt to substitute remote procedures. Statistical modeling will account for resultant variations in inter-visit follow-up intervals as necessary. Sites are permitted to perform telephone assessment of clinical elements not affected by telephone collection such as participant demographics, medical and family history, Clinical Dementia Rating Scale, and Geriatric Depression Score [44, 45] for the purposes of shortening the duration of face-to-face clinical assessments or when scheduled clinical visits are delayed by study site shutdown. Sites are also encouraged to contact all participants during study shutdown to reassure them that the MarkVCID studies remain active and will resume when fully safe.

The overarching goal of MarkVCID is to facilitate future multi-site observational studies and treatment trials for SVD-related VCID. The full fluid-based assays and neuroimaging protocols as well as the results of the ongoing fluid- and imaging-based biomarker kit instrumental and biological validation studies will be made available to the VCID community to serve as the basis for such trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

[1]. Montine TJ, Koroshetz WJ, Babcock D, Dickson DW, Galpern WR, Glymour MM, et al. Recommendations of the Alzheimer's disease-related dementias conference. Neurology. 2014;83:851–860. [PubMed: 25080517]

[2]. Boyle PA, Yu L, Wilson RS, Leurgans SE, Schneider JA, Bennett DA. Person-specific contribution of neuropathologies to cognitive loss in old age. Ann Neurol. 2018;83:74–83. [PubMed: 29244218]

[3]. National Institutes of Health. RFA-NS-16–020: Small Vessel Vascular Contributions to Cognitive Impairment and Dementia (VCID) Biomarkers Development Projects (UH2/UH3). 2016. https://grants.nih.gov/grants/guide/rfa-files/RFA-NS-16-020.html. Date accessed: January 6, 2020.

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[4]. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource. Silver Spring, MD: Food and Drug Administration/National Institutes of Health; 2016.

[5]. Vilar-Bergua A, Riba-Llena I, Nafria C, Bustamante A, Llombart V, Delgado P, et al. Blood and CSF biomarkers in brain subcortical ischemic vascular disease: Involved pathways and clinical applicability. J Cereb Blood Flow Metab. 2016;36:55–71. [PubMed: 25899297]

[6]. Rosenberg GA, Wallin A, Wardlaw JM, Markus HS, Montaner J, Wolfson L, et al. Consensus statement for diagnosis of subcortical small vessel disease. J Cereb Blood Flow Metab. 2016;36:6–25. [PubMed: 26198175]

[7]. Altendahl M, Maillard P, Harvey D, Cotter D, Walters S, Wolf A, et al. An IL-18-centered inflammatory network as a biomarker for cerebral white matter injury. PLoS One. 2020;15:e0227835.

[8]. Lu H, Kashani AH, Arfanakis K, Caprihan A, DeCarli C, Gold BT, et al. MarkVCID Cerebral small vessel consortium: II. Neuroimaging protocols (under review, May 2020).

[9]. Zhang X, Matsui K, Krohmal B, Zeid AA, Muthuswamy V, Koo YM, et al. Attitudes towards transfers of human tissue samples across borders: an international survey of researchers and policy makers in five countries. BMC Med Ethics. 2010;11:16. [PubMed: 20843366]

[10]. Malin B, Loukides G, Benitez K, Clayton EW. Identifiability in biobanks: models, measures, and mitigation strategies. Hum Genet. 2011;130:383–392. [PubMed: 21739176]

[11]. Tudur Smith C, Hopkins C, Sydes MR, Woolfall K, Clarke M, Murray G, et al. How should individual participant data (IPD) from publicly funded clinical trials be shared? BMC Med. 2015;13:298. [PubMed: 26675031]

[12]. Blasimme A, Fadda M, Schneider M, Vayena E. Data Sharing For Precision Medicine: Policy Lessons And Future Directions. Health Aff (Millwood). 2018;37:702–709. [PubMed: 29733719]

[13]. Evans BJ, Jarvik GP. Impact of HIPAA's minimum necessary standard on genomic data sharing. Genet Med. 2018;20:531–535. [PubMed: 28914268]

[14]. National Institutes of Health. Final NIH Statement on Sharing Research Data. 2003. https://grants.nih.gov/grants/policy/data_sharing/. Date accessed: January 6, 2020.

[15]. The Genetic Information Nondiscrimination Act. The Genetic Information Nondiscrimination Act: A First Step Toward Protecting Americans From Misuse of Genetic Information. J Oncol Pract. 2009;5:40–41. [PubMed: 29447549]

[16]. Equal Employment Opportunity Commission. Genetic Information Nondiscrimination Act. Final rule. Fed Regist. 2016;81:31143–31159. [PubMed: 27192741]

[17]. Natelson Love M, Fathallah-Shaykh H. Network for excellence in neuroscience clinical trials: NeuroNEXT. JAMA Neurol. 2013;70:1227–1228. [PubMed: 23921561]

[18]. Ramos EM, Din-Lovinescu C, Bookman EB, McNeil LJ, Baker CC, Godynskiy G, et al. A mechanism for controlled access to GWAS data: experience of the GAIN Data Access Committee. Am J Hum Genet. 2013;92:479–488. [PubMed: 23561843]

[19]. Wong KM, Langlais K, Tobias GS, Fletcher-Hoppe C, Krasnewich D, Leeds HS, et al. The dbGaP data browser: a new tool for browsing dbGaP controlled-access genomic data. Nucleic Acids Res. 2017;45:D819–D826. [PubMed: 27899644]

[20]. Steinsbekk KS, Ursin LO, Skolbekken JA, Solberg B. We're not in it for the money-lay people's moral intuitions on commercial use of 'their' biobank. Med Health Care Philos. 2013;16:151–162. [PubMed: 22028241]

[21]. Prusaczyk B, Cherney SM, Carpenter CR, DuBois JM. Informed Consent to Research with Cognitively Impaired Adults: Transdisciplinary Challenges and Opportunities. Clin Gerontol. 2017;40:63–73. [PubMed: 28452628]

[22]. Besser L, Kukull W, Knopman DS, Chui H, Galasko D, Weintraub S, et al. Version 3 of the National Alzheimer's Coordinating Center's Uniform Data Set. Alzheimer Dis Assoc Disord. 2018;32:351–358. [PubMed: 30376508]

[23]. National Institute on Aging Alzheimer's Centers Program. National Alzheimer's Coordinating Center. 2010. https://www.alz.washington.edu/. Date accessed: August 16, 2019.

[24]. Hollingshead AB. Two Factor Index of Social Position. New Haven, Connecticut: Yale University; 1957.

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[25]. Verghese J, Lipton RB, Hall CB, Kuslansky G, Katz MJ, Buschke H. Abnormality of gait as a predictor of non-Alzheimer's dementia. N Engl J Med. 2002;347:1761–1768. [PubMed: 12456852]

[26]. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol. 1994;49:M85–94. [PubMed: 8126356]

[27]. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology. 1993;43:2412–2414.

[28]. Sheikh JIY JA Geriatric Depression Scale (GDS). Recent evidence and development of a shorter version. In: Brink TL, editor. Clinical Gerontology: A Guide to Assessment and Intervention. NY: The Haworth Press, Inc.; 1986. p. 165–173.

[29]. Weintraub S, Besser L, Dodge HH, Teylan M, Ferris S, Goldstein FC, et al. Version 3 of the Alzheimer Disease Centers' Neuropsychological Test Battery in the Uniform Data Set (UDS). Alzheimer Dis Assoc Disord. 2018;32:10–17. [PubMed: 29240561]

[30]. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. AJR Am J Roentgenol. 1987;149:351–356. [PubMed: 3496763]

[31]. Knight Alzheimer Disease Research Center. CDR Dementia Staging Instrument. https://knightadrc.wustl.edu/CDR/CDR.htm. Date accessed: January 6, 2020.

[32]. Wipfler P, Heikkinen A, Harrer A, Pilz G, Kunz A, Golaszewski SM, et al. Circadian rhythmicity of inflammatory serum parameters: a neglected issue in the search of biomarkers in multiple sclerosis. J Neurol. 2013;260:221–227. [PubMed: 22875099]

[33]. Dominguez-Rodriguez A, Abreu-Gonzalez P, Kaski JC. Inflammatory systemic biomarkers in setting acute coronary syndromes--effects of the diurnal variation. Curr Drug Targets. 2009;10:1001–1008. [PubMed: 19860643]

[34]. Devaraj S, Wang-Polagruto J, Polagruto J, Keen CL, Jialal I. High-fat, energy-dense, fast-food-style breakfast results in an increase in oxidative stress in metabolic syndrome. Metabolism. 2008;57:867–870. [PubMed: 18502272]

[35]. Bartlett JW, Frost C. Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. Ultrasound Obstet Gynecol. 2008;31:466–475. [PubMed: 18306169]

[36]. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1:307–310. [PubMed: 2868172]

[37]. Alzheimer's Disease Neuroimaging Initiative. Alzheimer's disease neuroimaging initiative. 2017. http://adni.loni.usc.edu/. Date accessed: January 6, 2020.

[38]. The National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site. NG00020 - NIA-LOAD GWAS. The National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site: University of Pennsylvania School of Medicine; 2016. https://www.niagads.org/datasets/ng00020. Date accessed: January 6, 2020.

[39]. Hansson O, Mikulskis A, Fagan AM, Teunissen C, Zetterberg H, Vanderstichele H, et al. The impact of preanalytical variables on measuring cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis: A review. Alzheimers Dement. 2018;14:1313–1333. [PubMed: 29940161]

[40]. del Campo M, Mollenhauer B, Bertolotto A, Engelborghs S, Hampel H, Simonsen AH, et al. Recommendations to standardize preanalytical confounding factors in Alzheimer's and Parkinson's disease cerebrospinal fluid biomarkers: an update. Biomark Med. 2012;6:419–430. [PubMed: 22917144]

[41]. O'Bryant SE, Gupta V, Henriksen K, Edwards M, Jeromin A, Lista S, et al. Guidelines for the standardization of preanalytic variables for blood-based biomarker studies in Alzheimer's disease research. Alzheimers Dement. 2015;11:549–560. [PubMed: 25282381]

[42]. Rozga M, Bittner T, Batrla R, Karl J. Preanalytical sample handling recommendations for Alzheimer's disease plasma biomarkers. Alzheimers Dement (Amst). 2019;11:291–300. [PubMed: 30984815]

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[43]. National Alzheimer's Coordinating Center. Biospecimen best practice guidelines for the Alzheimer's Disease Centers. University of Washington School of Public Health2014. https://www.alz.washington.edu/BiospecimenTaskForce.html. Date accessed: January 6, 2020.

[44]. Burke WJ, Roccaforte WH, Wengel SP, Conley DM, Potter JF. The reliability and validity of the Geriatric Depression Rating Scale administered by telephone. J Am Geriatr Soc. 1995;43:674–679. [PubMed: 7775729]

[45]. Randolph C, Williams JBW, Hannesdottir K, Eureyecko E, Langbaum JB, Tariot P, et al. Telephone administration of the CDR: Excellent agreement with face-to-face administration. Alzheimers Dement. 2014;10:P364.

[46]. Brandt J. The Hopkins Verbal Learning Test: Development of a new memory test with six equivalent forms. The Clinical Neuropsychologist. 1991;5:125–142.

[47]. Delis DC, Kramer JH, Kaplan E, Ober BA. California Verbal Learning Test-Second Edition (CVLT-II). San Antonio, TX: Psychological Corporation; 2000.

[48]. Gonzalez HM, Mungas D, Reed BR, Marshall S, Haan MN. A new verbal learning and memory test for English- and Spanish-speaking older people. J Int Neuropsychol Soc. 2001;7:544–555. [PubMed: 11459106]

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Participant Enrollment

- Project sites enroll diverse study cohorts using site-specific inclusion and exclusion criteria so as to provide generalizable validation data across a range of cognitive statuses, risk factor profiles, small vessel disease severities, and racial/ethnic characteristics representative of the diverse patient groups that might be enrolled in a future VCID trial. MarkVCID project sites include both prospectively enrolling centers and centers providing extant data and samples from preexisting community- and population-based studies.
- With approval of local institutional review boards, all sites incorporate MarkVCID consensus language into their study documents and informed consent agreements. The consensus language asks prospectively enrolled participants to consent to unrestricted access to their data and samples for research analysis within and outside MarkVCID. The data are transferred and stored as a de-identified data set as defined by the Health Insurance Portability and Accountability Act Privacy Rule.
- Similar human subject protection and informed consent language serve as the basis for MarkVCID Research Agreements that act as contracts and data/biospecimen sharing agreements across the consortium.

Clinical and Cognitive Data

- Clinical and cognitive data are collected across prospectively enrolling project sites using common MarkVCID instruments. The clinical data elements are modified from study protocols already in use such as the Alzheimer's Disease Center program Uniform Data Set Version 3 (UDS3), with additional focus on VCID-related items such as prior stroke and cardiovascular disease, vascular risk factors, focal neurologic findings, and blood testing for vascular risk markers and kidney function including hemoglobin A1c, cholesterol subtypes, triglycerides, and creatinine.
- Cognitive assessments and rating instruments include the Clinical Dementia Rating Scale, Geriatric Depression Scale, and most of the UDS3 neuropsychological battery. The cognitive testing requires approximately 60 to 90 minutes.
- Study staff at the prospectively recruiting sites undergo formalized training in all measures and review of their first three UDS3 administrations by the coordinating center.

Collection and Handling of Fluid Samples

- Fluid sample types collected for MarkVCID biomarker kits are serum, EDTA-plasma, platelet-poor plasma, and cerebrospinal fluid (CSF) with additional collection of packed cells to allow future DNA extraction and analyses.

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- MarkVCID fluid guidelines to minimize variability include fasting morning fluid collections, rapid processing, standardized handling and storage, and avoidance of CSF contact with polystyrene.

Instrumental Validation for Fluid-based Biomarkers

- Instrumental validation of MarkVCID fluid-based biomarkers is operationally defined as determination of intra-plate and inter-plate repeatability, inter-site reproducibility, and test-retest repeatability. MarkVCID study participants both with and without advanced small vessel disease are selected for these determinations to assess instrumental validity across the full biomarker assay range.
- Intra- and inter-plate repeatability is determined by repeat assays of single split fluid samples performed at individual sites. Inter-site reproducibility is determined by assays of split samples distributed to multiple sites. Test-retest repeatability is determined by assay of three samples acquired from the same individual, collected at least five days apart over a 30-day period and assayed on a single plate.

The MarkVCID protocols are designed to allow direct translation of the biomarker validation results to multicenter trials. They also provide a template for outside groups to perform analyses using identical methods and therefore allow direct comparison of results across studies and centers. All MarkVCID protocols are available to the biomedical community and intended to be shared.

In addition to the instrumental validation procedures described here, each of the MarkVCID kits will undergo biological validation to determine whether the candidate biomarker measures important aspects of VCID such as cognitive function. Analytic methods and results of these validation studies for the 11 MarkVCID biomarker kits will be published separately. The results of this rigorous validation process will ultimately determine each kit’s potential usefulness for multicenter interventional trials aimed at preventing or treating small vessel disease related VCID.

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Table 1

Summary of standardized informed consent language

See https://markvcid.partners.org/consortium-protocols-resources for detailed language under each section

Principles for informed consent language
1. Types of subjects
• Site-specific inclusion and exclusion criteria
2. Areas for which research data/biospecimens can be used
• Purpose of research and repository
• Permission to use data/biospecimens for future research
• Permission for genetic analysis and creation of cell lines
• Sharing genetic data with banks and central repositories
3. Types of data/materials shared
• Collection of required imaging, clinical/cognitive data, and biospecimens
4. Privacy protection
• Deidentification of protected health information and biospecimens
5. Sharing biospecimens and data including sharing with commercial entities
• Sharing samples and data with universities, hospitals, commercial entities, government agencies, and not-for-profit organizations
• Disclosure that biosamples and data cannot be sold for profit though could be used for future tests that might be used for profit
• Maintain safeguards to protect participant privacy
6. Time requirement for retention of data/samples
• Indefinite storage of biospecimens and data
• Participant right to withdraw unused samples/data from repository
7. Receiving research results
• Option for participants to receive or not receive medically significant results from biospecimens or data resulting from the study
• Participant responsibility for costs for clinically indicated tests and follow-up care
8. Loss of privacy risk language
• Potential risk of loss of privacy
• Encoding of samples and Federal law protection
• Unforeseeable technological advances that could link data to participant

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Table 2

Selected demographic, clinical, and cognitive measures

See https://markvcid.partners.org/consortium-protocols-resources for full information.

Category	Uniform Data Set 3.0, National Alzheimer's Coordinating Center (UDS3, NACC) elements	MarkVCID added measures
Demographics & related elements	Year of birth, sex, race and ethnicity, primary language, handedness, marital status, living situation, level of independence, zip code of primary residence, education	Age at registration, self-reported fluency in English, occupational status
Medical/Neurological/ Psychiatric history	History of cigarette smoking, cardiovascular disease, cerebrovascular disease history, neurologic conditions, medical conditions, substance abuse, psychiatric conditions, medical history	Nature and timing of cerebrovascular disease history
Family history	Acquired cognitive impairment/dementia/Alzheimer's Disease	Stroke/Transient ischemic attack (TIA)
General physical measures	Vital signs, additional physical observations (vision and hearing)	Short Physical Performance Battery (SPPB)
Neurological exam	Parkinsonian features, cerebrovascular features, other findings	
Cognitive diagnosis	Syndromic Diagnosis, primary Etiological Diagnoses, related Etiological Diagnoses	
Montreal Cognitive Assessment (MoCA)	Language of test administration, visuospatial/executive, language naming, memory, attention, language repetition and fluency, abstraction, delayed recall, orientation	
Neuropsychological Testing Battery	Language of test administration, craft story 21 recall, number span forward and back, Category fluency (animals) verbal fluency [words with F (English) or P (Spanish)], trail making test A and B, Multilingual Naming Test (MINT)	Word list learning (choice of Hopkins Verbal Learning test [HVLT] [46], California Verbal Learning Test-2 [CVLT-2] [47], Spanish English Verbal Learning Test [SEVLT] [48])
Overall Function	Clinical Dementia Rating (CDR)	
Depression	Geriatric Depression Scale (GDS)	
Laboratory Tests (required)	-	HbA1c; serum, HDL, LDL cholesterol (fasting); triglycerides; creatinine
Laboratory Tests (optional)	-	HS-CRP, blood sugar (fasting), homocysteine (fasting), genetics

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Table 3

Summary of MarkVCID fluid biomarker instrumental validation

Instrumental validation step	Number of subjects	Timepoints
Intra-plate and inter-plate repeatability	Eight per site performing fluid assays	N/A
Inter-site reproducibility	40 (plasma) / 20 (platelet poor plasma) / 20 (cerebrospinal) shared across all participating sites	N/A
Test-retest repeatability	60 (10 per prospectively enrolling site)	Three blood draws within 30 days, at least five days apart