MarkVCID MRI Cerebrovascular Reactivity (CVR) Biomarker Kit Protocol

1. Executive summary and timeline

The proposed biomarkers

Cerebrovascular reactivity (CVR) Kit. Primary Biomarker Category: Susceptibility/Risk. Secondary Biomarker Category: Mechanism of Action

Hypothesis to be tested

Instrumental validation

1) Test-retest using separate CVR scans on the same individual

Each participating site (including the JHU site) will ask at least 6 patients to return for repeat CVR determination within 1 week of initial MRI/CO2 challenge. Power calculation: According to our data collected in the UH2 phase, correlation coefficient between scans was 0.97. Therefore, assuming that true test-retest intraclass correlation coefficient is 0.9, we will require 23 subjects (surpassed by 6 per site x 4 sites) to have 80% power to detect a difference from the null hypothesis of ICC=0.7.

2) Inter-rater reliability showing that different raters calculate the same result for CVR when analyzing the same scan.

We will use the baseline MRI/CO2 challenges from the same 6 subjects per site, circulating the data to other sites to determine inter-rater reliability. We have fully automated our CVR data processing. Thus, we expect that there will not be an inter-rater dependence of the CVR results. That is, power is expected to be close to 1 (for automated scripts run on the same scan).

Biological validation

Individual-site data: At each site, cross-sectionally whole-brain CVR will be positively associated with general cognitive function, after accounting for age, sex, and education. The primary cognitive outcome measure will be the Montreal Cognitive Assessment for Dementia (MoCA). Secondary cognitive measures will be the global z-score across all domains (average of individual z-scores for memory, executive function, processing speed, and fluency domains and domain-specific measures. For example, based on our UH2 data, we hypothesize that cognitive scores combining language and executive function domains will show an association with CVR. Whole-brain CVR will also be associated with clinical diagnosis of MCI and dementia. As a secondary hypothesis, for sites that have amyloid/tau measures (either through CSF or PET imaging), CVR will be independently associated with cognitive scores and clinical diagnosis controlling for CSF or PET Aβ42/tau measures. For sites that conduct test-retest reproducibility assessment, inter-session CoV of whole-brain CVR will be between 5-10%, consistent with the JHU UH2-phase test results.

Longitudinal multi-site data: We will calculate the rate of CVR decline as (CVR at follow-up – CVR at baseline)/time interval. A rate of decline will also be calculated for MoCA and z-scores of cognitive measures, e.g. general cognitive score, the executive function domain score, and the language domain score. We hypothesize that, across individuals, the rate of cognitive decline will be significantly associated with the rate of CVR decline. Using the average rate of CVR decline and standard errors obtained in the longitudinal data analysis, we will estimate the minimal sample size needed to detect a 25% difference in CVR decline rate between two arms of a clinical trial.

SVD biology and outcome the biomarker measures

The brain's ability during performance of a cognitive task is a dynamic process and requires small blood vessels to dilate or constrict in real time to adjust blood flow in a region-specific manner. This coupling is sometimes referred to as the "neurovascular unit". A loss or diminishment of such capacity is expected to negatively impact neural function and therefore cognition. CVR provides a quantitative evaluation of the status of the neurovascular unit. It measures the ability of vessels to react to vasoactive challenges.

Timeline for validation of the biomarker

1-3 months: Set up CVR data acquisition and analysis pipeline at all participating sites. Conduct training (if necessary) and quality control.

4-12 months: Collect data from 1/3 of the sample and conduct interim analysis for consortium meeting and for evaluation of progress.

12-30 months: Continue the collection of data.

31-36 months: Each site conducts their own data analysis to validate the CVR biomarker. The proposing site conducts the multi-site analysis.

2. Brief description of biomarker kit

Description of final biomarker

The brain's ability during performance of a cognitive task is a dynamic process and requires small blood vessels to dilate or constrict in real time to adjust blood flow in a region-specific manner. This coupling is sometimes referred to as the "neurovascular unit". A loss or diminishment of such capacity is expected to negatively impact neural function and therefore cognition. CVR provides a quantitative evaluation of the status of the neurovascular unit. It measures the ability of vessels to react to vasoactive challenges. For the validation in the UH3 phase, we will focus on the whole-brain CVR, with regional CVR as secondary biomarkers.

Kit components

Materials and protocols provided for cross-site testing: Our CVR kit contains a hardware component and a software component. We will provide the hardware component in a FedEx box of approximately 18"x18", which contains most of the parts needed for the CVR experiment, i.e. 1 ETCO2 monitor , 1 CO2 sensor, 1 Douglas bag,1 three-way valve, 1 gas delivery tube, 1 two-way non-breathing valve, 1 U-shape breathe tube, 20 white nose clips, 10 blue nose clips, 20 mouth pieces, 20 CO2 sampling lines extension, 1 Dehumidification tubing, 1 sample line (male luer), 1 linecord, 1 gas delivery tube(from tank to bag), 1 blue cuff, 1 watch, 1 timer, 20 experiment recording sheets, and 20 subject rating sheets. The only part that needs to be ordered by the local site is the gas tank, for which we will provide the vendor name and item number. The software component includes MRI protocol, MATLAB analysis scripts, and step-by-step instructions, which are typically transferred through email. With these hardware and software components, each site will be able to fully and independently carry out all necessary experiments and analyses, and prepare results to report back to the Steering Committee, the External Advisory Committee, and the NINDS.

Once the site receives the FedEx box, Dr. Lu and his staff will visit the site, help set up the CVR system, train the site staff through 1 or 2 actual CVR experiments, demonstrate the analysis methods, and show the CVR results. Dr. Lu has visited all participating sites mentioned below.

For necessary clinical data, each site should have at least a clinical diagnosis and Cognitive batteries of memory, executive function, processing speed, and fluency domains (for each time point at which CVR is acquired). All test scores should be converted to z-scores before statistical analysis.

3. Participating sites

Four VCID sites, JHU, UKY, UNM, and USC, have indicated their participations in the cross-site testing of the CVR MRI biomarker. Table 1 summarizes the sample size and participant characteristics. A total of 360 participants will be studied. Of these, 300 participants will have a follow-up CVR MRI at 12 and 24 months. Cohorts at different sites have complementary characteristics. The USC cohort is primarily Latino subjects, which is important given the high prevalence of cardiovascular diseases in the Latino population. The UNM cohort represents the severe end of the spectrum of small vessel disease with many having clinically diagnosed Binswanger disease. The UKY and JHU cohorts represent typical racial/ethnic distributions of the U.S. population with the JHU cohort having the highest representation of African American among all sites in the VCID consortium. We can therefore test if CVR is a useful biomarker in a range of VCID spectrum parts and in different racial/ethnic categories.

	JHU	**UKY**	**UNM**	**USC**
Number of subjects with cross-sectional data	75	120	75	90
Number of subjects with longitudinal	75	120	75	30

follow-up				
Mean age	70	75	64	70
Subject characteristics	50 MCI/ 25 Normal	40 MCI with SVID/ 40 Normal with SVID/ 40 Normal	53 patients/ 22 Normal	MCI or Normal

We will focus on the validity of the CVR kit as a biomarker in small vessel disease. Thus, participants with a history of stroke, moyamoya disease, symptomatic stenosis, and vascular malformation will be excluded.

4. Protocol for MRI acquisition OR fluid sample acquisition

Magnetic Resonance Imaging (MRI)

MRI at JHU will be performed on a research-dedicated 3 Tesla MR system (Philips Healthcare) housed in the F.M. Kirby Center for Functional Brain Imaging. A 32-channel head coil will be used for receiving.

Cerebrovascular Reactivity (CVR)

CVR will be assessed using 5% CO_2-breathing while continuously acquiring MR images. This method has been extensively used in our laboratory and the UH2 data shown in the Transition Report were acquired using this method. Briefly, the CO_2 air (5% CO_2, 21% O_2 and 74% N_2) will be administered via an air bag (Figure 1) with a valve to switch between room air and CO_2 air in the bag. A mouth piece and a nose clip will be used to achieve mouth-only breathing. A research staff member will be inside the magnet room throughout the experiment to switch the valve and to monitor the subject. Figure 1a and b show the illustration and a picture of the setup, respectively. Physiologic parameters, including end-tidal (Et) CO_2 and breathing rate will be monitored and recorded during the experiments. Figure 1c shows an example of CO_2 recording measured by the capnography, illustrating the effect of CO_2 breathing on lung and blood CO_2 content. Since the Et-CO_2 is essentially the input function to the brain vasculature, it is critical to measure this trace as we then know how much stimulation the blood vessel receives.

MR images will be acquired continuously during the CVR scan using a BOLD protocol that we have standardized across VCID sites: gradient echo EPI, voxel size=3.4X3.4x3.8 mm^3, matrix=64x64x36, Number of slices=34-36 (depending on MRI scanner model), TR=1500ms, TE=21ms, flip angle=90°, and number of volumes=281 (plus 6 dummy volumes). The subject will breathe 15s room-air, 50s gas mixture, 70s room-air, 50s gas mixture, 70s room-air, 50s gas mixture, and 115s room-air. The CVR scan lasts for approximately 7 minutes. The additional setup time to fit the mouth-piece and nose-clip is expected to be less than 3 minutes.

Figure 1: CVR measurement with 5% CO_2 breathing. **(a)** Diagram illustrating the components of the system. **(b)** A picture showing a subject with the apparatus. **(c)** Capnography recording showing the partial pressure of CO_2 in the sampled air. The black curve shows the raw recording, with high-frequency fluctuation corresponding to breathe-in and breathe-out. Within each breath, the peak appears at the end of exhalation indicating the CO_2 concentration in the lung (thus the name "end-tidal CO_2"). The trough is obtained at the end of inhalation indicating the CO_2 concentration in the inhaled air. In this example, the subject inhaled 1 minute of room-air interleaved with 1 minutes of CO_2, and repeated four times.

After the CVR scan while still inside the MRI room, the subject will be asked to provide a rating (from 1

to 10) on the comfort level of their breathing during the CVR scan. A rating will also be obtained after the no-mouthpiece MRI sequences to serve as a reference. A rating of overall experience of the MRI session will also be obtained after all MRI procedures have been completed. These ratings will be collected for each participant at every site.

Each participating site (including the JHU site) will ask at least 6 patients to return for repeat CVR determination within 1 week of initial MRI/CO2 challenge.

5. Additional data (clinical/cognitive etc.) required for analysis

For necessary clinical data, each site should have at least a clinical diagnosis, MoCA, and Cognitive batteries of memory, executive function, processing speed, and fluency domains. All test scores should be converted to z-scores before statistical analysis.

6. Protocol for MRI analysis OR fluid analysis

CVR data will be processed using a general linear model (SPM, University College London, UK) similar to a typical fMRI scan, except that the regressor will be the Et-CO2 time course rather than the fMRI paradigm. Absolute CVR in units of %BOLD signal change per mmHg of Et-CO_2 change (%BOLD/mmHg CO_2) will be obtained. The primary outcome variable of the CVR measure is the whole-brain CVR value, with lobar CVR as secondary variables. The proposing site will transfer the MATLAB analysis scripts and step-by-step instructions to the participating sites through email. As an ongoing effort, we are also in the process of implementing a cloud-based platform for these scripts, which, if successful, will replace the email transfers. With these items, each site will be able to fully and independently carry out all necessary experiments and analyses.

7. Step-by-step analytic plan

Instrumental validation

1. Test-retest using separate CVR scans on the same individual

Intraclass-correlation coefficient (ICC) between whole-brain CVR values measured in two CVR scans on the same individual will be calculated. Additionally, coefficient-of-variation (CoV) of the whole-brain CVR values across scans will be computed as standard deviation across scans divided by mean. We expect that the correlation between CVR measured using two scans performed on the same individual will be >0.7. We further hypothesize that inter-scan CoV of CVR will be less than 7%.

2. Inter-rater reliability showing that different raters calculate the same result for CVR when analyzing the same scan.

Two independent raters will process the same CVR scan. We will use the baseline MRI/CO2 challenges from the same 6 subjects per site, circulating the data to other sites to determine inter-rater reliability. We have fully automated our CVR data processing. Thus, we expect that there will not be an inter-rater dependence of the CVR results. That is, power is expected to be close to 1 (for automated scripts run on the same scan).

Biological validation

Cross-sectional data. We hypothesize that the cross-sectional findings observed in the UH2 phase that, CVR measured by MRI is positively associated with cognitive function measured by the MoCA, will be reproduced at other sites. Descriptive statistics on CVR and cognitive scores will be summarized by mean, median, standard deviation and the 95% confidence intervals for each site. The association between the CVR measurement and the cognitive scores will be visualized using scatter plots. To test whether CVR is statistically significantly associated with general cognition, a multivariate linear regression will be performed with the general cognitive function score being the dependent variable, and the whole-brain CVR being the independent variable (done for data from each site). Age, sex, and education will be included as covariates. The primary cognitive function measure will be the MoCA. Secondary cognitive measures will be the global z-score (average of individual z-scores for memory, executive function, processing speed, and fluency domains and domain-specific measures. For example, based on our UH2 data, we hypothesize that cognitive scores combining language and

executive function domains will show an association with CVR. As a secondary analysis, for sites that have amyloid/tau measures (either through CSF or PET imaging), amyloid/tau will also be included as a covariate.

Hypothesized outcomes: At each site, cross-sectionally whole-brain CVR will be positively associated with general cognitive function, after accounting for age, sex, and education. Whole-brain CVR will also be associated with clinical diagnosis of MCI and dementia. We further hypothesize that composite cognitive scores combining language and executive function domains from the consortium's harmonized testing battery will show an association with CVR. As a secondary outcome, for sites that have amyloid/tau measures (either through CSF or PET imaging), CVR and Aβ42/tau will be independently associated with general cognitive score and clinical diagnosis.

3. Sample size calculation (individual site level)

Instrumental validation

1. Test-retest using separate CVR scans on the same individual

According to our data collected in the UH2 phase, correlation coefficient between scans was 0.97. Therefore, assuming that true test-retest intraclass correlation coefficient is 0.9, we will require 23 subjects (surpassed by 6 per site x 4 sites) to have 80% power to detect a difference from the null hypothesis of ICC=0.7.

2. Inter-rater reliability showing that different raters calculate the same result for CVR when analyzing the same scan.

Since we expect that the inter-rater reliability will effectively be zero (since no manual inputs are needed in the entire processing), our sample size of 6 at each site will be sufficient to verify our hypothesis.

Biological validation

The correlation coefficient between cognitive scores and CVR is expected to be at least 0.35 based on the primary data from the UH2 study. To calculate the power at each of the four sites for detecting a correlation coefficient from 0 under the null hypothesis to 0.35 under the alternative hypothesis, a variance inflation factor (VIF) is applied to account for the covariates in the multivariate regression, some of which have certain degrees of co-linearity with CVR (e.g. age). The VIF for adding covariates to a model has been shown to depend on the squared multiple correlation coefficient (R^2) relating the independent variables in a regression model. The R^2 relating CVR with age, sex and education is about 0.11 based the preliminary data from the UH2 study. We expect the R^2 relating CVR with age, sex, education and the potential amyloid measures are at most 0.15. In this case, the VIF is 0.18. Consequently, the effective sample sizes for univariate linear regression is 63 for JHU and for UNM, 101 for UKY and 76 for USC. Therefore, the power for detecting a correlation coefficient from 0 under the null hypothesis to 0.35 under the alternative hypothesis with two-sided type 1 error rate of 0.05 and taken into account the covariate adjustment are 81% for JHU and for UNM, 95% for UKY and 88% for USC.

3. Plan for longitudinal data collection analysis

Longitudinal data analysis. We will first verify that there is a significant difference between CVR measured at baseline and those measured at follow-up. Next, we will calculate the rate of CVR decline as (CVR at follow-up – CVR at baseline)/time interval. A rate of decline will also be calculated for cognitive scores. Then, we will conduct a multi-linear regression in which the rate of cognitive decline will be the dependent variable and the rate of CVR decline will be the independent variable, with age, gender, and education as covariates. We expect that, across individuals, the rate of cognitive decline will be significantly associated with the rate of CVR decline. We will conduct this analysis on a site-by-site basis as well as pooling data from all sites with a site index in the model. Finally, the average rate of CVR decline across all sites will be obtained which will provide a benchmark index for future clinical trials.

Estimation of minimal sample size for future clinical trials. At the end of the UH3 phase, we will also provide an estimation on how many patients need to be enrolled to observe a 25% change (by a drug) in CVR decline rate. Using the average rate of CVR decline and standard errors obtained in the longitudinal data analysis, we will estimate the minimal sample size needed to detect a 25% difference in CVR decline rate between two arms of a clinical trial. This type of biomarker assessment has been used in the ADNI study for AD biomarkers. We believe that such analyses are also important in small vessel biomarker investigations.

Hypothesized outcomes: We hypothesize that, across individuals, the rate of cognitive decline will be significantly associated with the rate of CVR decline.

4. Plan for reporting outcomes

Interim report: By 12 months into the UH3 phase, we anticipate that we will have collected data from 1/3 of the sample. We will then conduct an interim analysis for consortium meeting and for evaluation of progress. Due to smaller sample size at this stage, we anticipate that the reporting of the results will primarily be based on multi-site data, although we will also investigate single-site results.

Final report: By 30 months into the UH3 phase, we anticipate that the majority of the data have been collected. Each site will then conduct site-specific data analysis to validate the CVR biomarker. The proposing site conducts the multi-site analysis. Manuscript publications will be based on scientific contributions with approval from the consortium's Publications Subcommittee.

5. Plan for sharing data, samples/images, protocols

No IP issues are anticipated. The CO2 breathing apparatus, pulse sequence, and analysis methods have been published (Lu et al. Journal of Visualized Experiment, 94, 2014).

Findings from the study will be reported in scientific publications. We plan to disseminate the optimized imaging parameters and analysis methods for CVR to interested researchers as we have been doing in the past few years. The de-identified data acquired during the UH3 phase will be stored on the consortium data server, and will be shared with investigators in and outside the consortium based on policies established by and with approval from the Data Sharing Subcommittee.

Other samples/data, e.g. biofluid, clinical, and neuropsychological, obtained during the UH3 phase will be shared to researchers in academics, not-for-profits, and industry (for internal research only, for-profit use is not allowed) following the plan below:

- The sample and de-identified clinical data will be submitted to an NINDS-designated repository or stored locally
- The de-identified samples and clinical data will be securely stored at the repository or database
- No personal identifiers will be sent with samples and data
- The de-identified samples and clinical data can be distributed to scientists for use in research and teaching only, and as such the Repository does not return results to donors
- The de-identified samples and clinical data could be used for research in any type of disease or genetic factors, not just vascular contributions to cognitive impairment and dementia
- The de-identified samples (as well as their derivatives) and clinical data will be available for research and teaching purposes to hospitals, universities, not for profit research organizations, and commercial organizations, via NINDS-designated repositories
- The de-identified samples and clinical data will be used for research only, and will never be distributed for profit
- There is a risk that someone could use information from the sample submitted, via DNA, to identify the person from which it came if it were matched with another DNA sample provided by that person. However, any user of this sample must agree not to use it for that purpose.