MarkVCID2 MRI Cerebrovascular Reactivity (CVR)

Biomarker Kit Protocol

1. Brief description of the biomarker kit (including biological validation citation)

The proposed biomarkers

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.

Cerebrovascular reactivity (CVR) Kit. Primary Biomarker Category: Subject Selection. Secondary Biomarker Category: Study Outcome

Kit lead/site: Hanzhang Lu/Johns Hopkins University

Hypothesis to be tested

Baseline CVR can predict changes in general cognitive function as measured by MoCA score over 24 months, after accounting for age, sex, and education.

CVR changes over 24 months can predict changes in general cognitive function as measured by MoCA over 24 months.

Biological validation

Biological validation of the CVR kit has been completed in UH3 phase of the MarkVCID project (1). For biological validation, it was pre-specified (based on phase 1 single-site data) that each site should have a minimum of 75 participants, in order to provide sufficient power for the proposed analysis. Therefore, a total of 3 analysis sites and 263 subjects were included in the biological validation study, which were Johns Hopkins University (JHU, N=77), University of Texas Health Science Center at San Antonio (UTHSCSA, N=92), University of Kentucky and University of Southern California (UKY/USC, N=95). This met the NINDS's requirement of "testing the pre-specified hypothesis in a minimum of three sites". Below we present the results from these data.

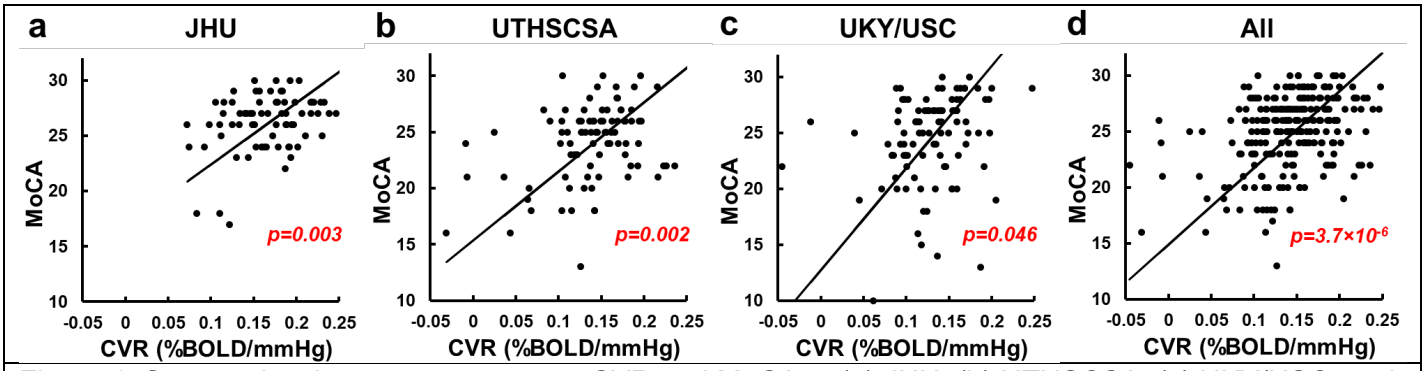

Figure 1: Scatter plots between gray matter CVR and MoCA at (a) JHU, (b) UTHSCSA, (c) UKY/USC, and (d) all sites. Each dot represents data from one individual.

As shown in Figure 1, whole-brain gray matter CVR showed a positive association with MoCA score after adjustment for age, sex, and education. Participants with higher CVR had higher MoCA scores. This relationship was reproduced at all analysis sites ($p < 0.05$ at all analysis sites), confirming our pre-specified hypothesis of the associations between CVR and global cognition.

As secondary analysis results, we examined the association between gray matter CVR and executive function, which is the primary cognitive domain affected by SVD and VCID. Our results showed that higher gray-matter CVR was significantly associated with better executive function, indicated by higher executive function (measured by item response theory (IRT)) score ($\beta = 2.87$, $p = 0.003$).

We then tested whether the associations between CVR and cognition were independent of other measures of vascular health, including vascular risk factors (VRS) and Fazekas score. The results are summarized in Table 3. It was found that higher CVR ($p < 0.001$, Table 3) and lower VRS ($p = 0.038$, Table 3), but not Fazekas score ($p = 0.30$, table 2), were independently associated with better MoCA scores after adjustment for age, sex, education, and site. Similarly, the executive function IRT score was significantly related to CVR ($p = 0.003$, Table 3) and VRS ($p = 0.004$, Table 3), but not Fazekas score ($p = 0.92$, Table 3), after adjustment for age, sex, education, and site. We also conducted additional analyses to examine the relationship between CVR and VRS/Fazekas score and observed that CVR was not associated with

VRS (p = 0.99) or Fazekas score (p = 0.70) after controlling for age, sex, education, and site. These findings suggested that CVR adds additional predictive power to classic vascular risks in evaluating relationships with cognition in SVD/VCID patients.

2. Summary of kit instrumental validation results (including instrumental validation citation)

Instrumental validation of the CVR kit has been completed in UH2 phase of the MarkVCID project, and the results have been published (doi: 10.1016/j.neuroimage.2021.118754.)(2). The results are summarized below:

a. Inter-rater reliability of CVR

The objective of this study was to investigate whether different raters reported consistent CVR results obtained in the same sample. This sample included 30 participants imaged with a harmonized CO2-CVR protocol at four different sites (Sites 1, 2, 3, and 4)(3). Table 1 lists demographic information of the participants. The 30 CO2-CVR datasets were sent to all five participating sites (Sites 1, 2, 3, 4, and 5) for analysis. Written instructions and a webinar were given to the investigators from each participating site for training. One rater from each site analyzed the datasets independently using the CVR-MRICloud processing pipeline (4) and shared outputs with the JHU site (site 1), resulting in 5 sites × 30 subjects CVR measures.

Table 1. Scanner and demographic information of the study.

	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**
Inter-rater reliability					
Scanner	Philips Achieva	Siemens Trio	Siemens Prisma	Siemens Trio	--
N	15	6	7	2	--
M/F	7M/8F	2M/4F	2M/5F	0M/2F	--
Age	70±5	72±6	69±4	78±4	--
Inter-scanner reproducibility					
Scanner	Philips Achieva and Siemens Prisma	--	--	--	--
N	10	--	--	--	--
M/F	7/3	--	--	--	--
Age (y)	24±2	--	--	--	--
Test-retest repeatability					
Scanner	Philips Achieva	Siemens	Siemens Prisma	--	Siemens
N	6	4	4	--	5
M/F	0M/6F	1M/3F	1M/3F	--	1M/4F
Age (y)	64±6	79±3	75±5	--	68±4

Figure 2 illustrates the whole-brain CVR values of 30 individuals processed by independent raters of the five participating sites. Visual inspection suggested good agreement across the sites. Inter-rater CoV was 0.08 ± 0.08% for whole-brain CVR values, and ranged from 0.16 % to 0.88 % in major brain regions. ICC_{AA} of whole-brain CVR measures generated by the five sites was found to be 0.9999, $p<0.0001$, indicating excellent reliability, i.e. a very strong agreement, between the different raters (i.e. sites) when using the CVR-MRICloud analysis pipeline on the same CO2-CVR datasets. ICC_{AA} of regional CVR values generated by the five sites ranged from 0.9959 to 0.9999 (see Table 2).

Table 2: Summary of CoV and ICC in all three instrumental validation studies.

	Whole-brain	Frontal	Parietal	Temporal	Limbic	Occipital	Insula	BasalGang	Thalamus
Inter-rater reliability									
CoV (%)	0.08±0.08	0.34±0.95	0.50±1.56	0.49±1.03	0.33±0.35	0.88±0.92	0.21±0.21	0.22±0.37	0.16±0.11
ICC_{AA}	0.9999	0.9977	0.9976	0.9995	0.9970	0.9999	0.9999	0.9999	0.9997
Inter-scanner reproducibility									

CoV (%)	6.90±5.08	7.99±5.93	8.21±6.61	7.53±5.56	8.46±7.87	7.92±6.96	12.71±13.32	10.27±9.56	4.69±4.39
ICC_C	0.8498	0.9185	0.8528	0.8052	0.8872	0.8068	0.8364	0.9175	0.8687

Test-retest repeatability									
CoV (%)	18.29±17.12	16.58±14.14	18.79±11.39	17.86±15.17	18.51±17.30	19.52±13.25	18.45±17.96	18.50±20.46	18.71±18.03
ICC_{AA} [d.]	0.6498	0.7785	0.7152	0.7100	0.7429	0.6480	0.7323	0.7716	0.7453

b. Inter-scanner reproducibility of CVR

To evaluate the reproducibility of CO2-CVR on different MRI platforms, a cross-vendor comparison was performed at the JHU site (Site 1). Ten healthy subjects (3 females, age 23.9±2.4y) participated in this study (Table 1). Each subject was scanned on a Philips (Achieva) and a Siemens (Prisma) 3 T scanner within a 2.5-hour period. On each scanner, CVR scans were performed twice with repositioning. The order of the scanners was randomized across subjects. The imaging acquisition and analysis protocol followed that described earlier, resulting in 2 vendors x 10 subjects x 2 repetitions CVR measures. To reduce the effects of physiological variations, the CVR values were further corrected for basal and ΔEtCO2 differences between repetitions and across the scanners using the association between them established previously

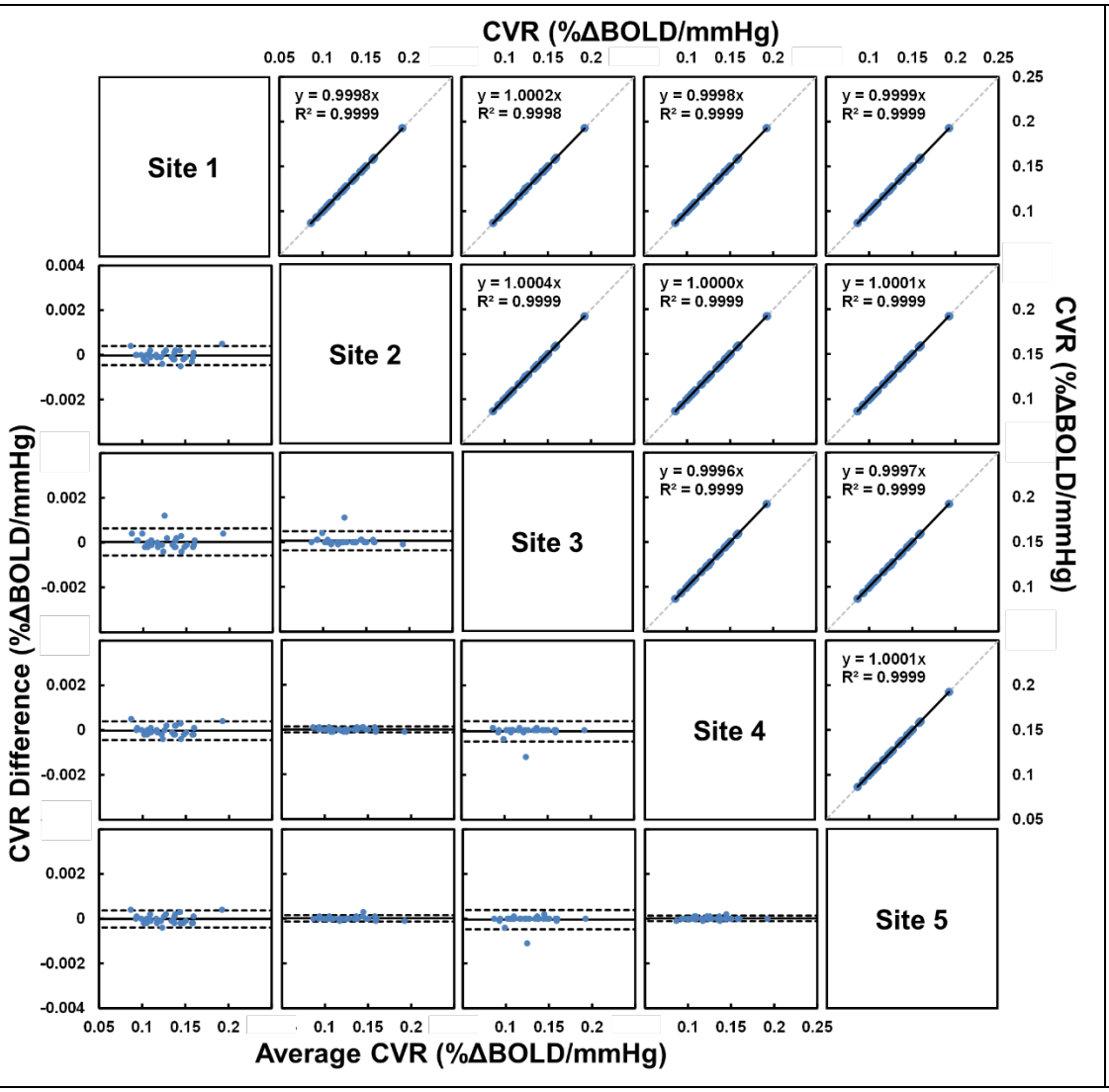

Figure 2. Comparisons of whole-brain CVR obtained from five different sites in the inter-rater reliability study. Scatterplots are shown in the upper triangle panel with linear regression line in black and identity line as dashed gray line. Bland-Altman plots are shown in the lower triangle panel. Each blue dot represents one subject.

Figure 3a displays CVR images from a representative subject, illustrating reproducible CVR maps. Scatter plot and Bland-Altman plot of whole-brain CVR for 10 individuals imaged at two different scanners are shown in Figure 3b. Visual inspection suggested good agreement between the scanners. Mean whole-brain CVR of the 10 healthy young subjects was 0.133±0.029 %ΔBOLD/mmHg and 0.126±0.025 % ΔBOLD/mmHg from the Philips scanner and Siemen scanner, respectively, with no significant difference between them (p=0.16). Inter-scanner CoV was 6.90 ± 5.08% for whole-brain CVR values, and ranged from 4.69 % to 12.71 % in major brain regions. ICC_C of whole-brain CVR measures from the two scanners was found to be 0.8498, p=0.0005. ICC_C of regional CVR values between two scanners ranged from 0.8052 to 0.9185 (see Table 2), indicating good to excellent reproducibility.

Additionally, same-day inter-session (with repositioning) analysis in the 10 subjects showed that for

whole-brain CVR measures, inter-session CoV was 6.30±8.97% for the Philips scanner, and 7.39±7.03% for the Siemens scanner, with no significant difference between them (p=0.86). Inter-session ICC_{AA} was 0.8744, p<0.0001 for the Philips scanner, and 0.7781, p=0.001 for the Siemens scanner.

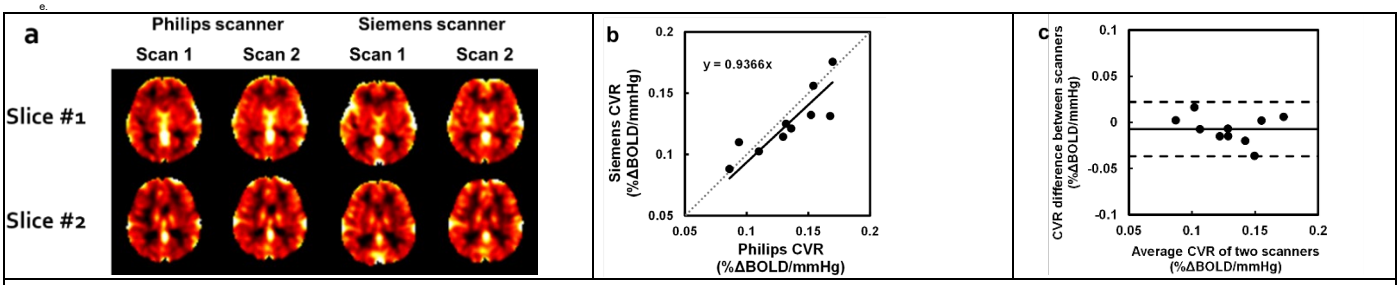

Figure 3. Reproducibility of CVR results between Philips and Siemens scanners. (a) Representative images from a subject. (b) Scatterplot of the whole-brain CVR measures with linear regression line in black and identity line as dashed gray line. (c) Bland-Altman plot of the whole-brain CVR measures. Each blue dot represents one subject.

c. Test-retest repeatability of CVR

To evaluate the test–retest repeatability of CO2-CVR on different days, four participating sites (Sites 1, 2, 3, and 5) recruited a total of 19 individuals. Each participant received two CO2-CVR scans using the same scanner and protocol with a gap 1 to 14 days. The number of the subjects from each site are shown in Table 1. Participating sites shared the de-identified data with the lead site for analysis. One subject from Site 2, two subjects from Site 3 and one subjects from Site 5 had technical issues with the CO2 trace recording during one of their 2 scans, so these four subjects were excluded from the test-retest analysis, resulting in 15 subjects x 2 repetitions CVR measures across 4 sites. Basal and ΔEtCO2 differences between the tests were also accounted for.

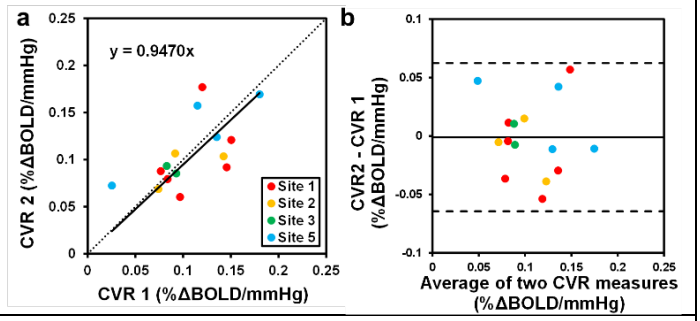

Figure 4: Comparisons of whole-brain CVR obtained from different days in the test-retest repeatability study (Study 3). (a) Scatterplot of the whole-brain CVR measures with linear regression line in black and identity line as dashed gray line. (b) Bland-Altman plot of the whole-brain CVR measures. Each blue dot represents one subject. Each color represents one participating site.

Figure 4 illustrates test and retest whole-brain CVR measures in 15 subjects across 4 sites. Mean whole-brain CVR of the 15 elderly subjects was 0.107±0.039 %ΔBOLD/mmHg and 0.106±0.036 % ΔBOLD/mmHg from the test-retest scans, respectively, with no significant difference between them (p=0.90). Test-retest CoV was 18.29 ± 17.12% for whole-brain CVR values, and ranged from 16.58 % to 19.52 % in major brain regions. ICC_{AA} between test and retest whole-brain CVR measures was found to be 0.6498, p=0.0041. ICC_{AA} of regional CVR values between the two measures ranged from 0.6480 to 0.7785 (see Table 2), indicating moderate to good reproducibility. There was not a site effect in the CoV values for the four sites.

3. Protocol for image acquisition

Magnetic Resonance Imaging (MRI)

MRI will be performed on 3 Tesla MR systems at each site following the MarkVCID neuroimaging protocols (3). 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.

Cerebrovascular Reactivity (CVR)

CVR will be assessed using 5% CO_2-breathing while continuously acquiring MR images. Briefly, the CO_2

air (5% CO_2, 21% O_2 and 74% N_2) will be administered via an air bag (Figure 5) 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 5a 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 5c 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. The Et-CO2 file should be considered part of the CVR data and be stored. The Et-CO2 file format will be dependent on the CO2 monitor used. One will need to refer to the specific CO2 monitor's user manual to understand the file format and how to read the file.

Figure 5: 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.

4. Additional data collection required for analysis

For necessary clinical data, each site should have at least age, sex, education, and MoCA score. All test scores should be converted to z-scores before statistical analysis.

5. Protocol for image processing

Each site will upload the CVR data to the Globus database, and all the CVR data will be processed centrally by the kit leading site.

CVR data processing will be performed using a cloud-based online processing tool referred to as CVR-MRICloud (4). With this cloud-based processing tool, the users will upload the de-identified CVR data files to the cloud server, including the BOLD image files, the CO2 trace file (after manually trimmed to remove extra recording periods before the mouthpiece was put on and after it was taken off), and the T1-MPRAGE image (average of the ME-MPRAGE images across all echoes) which allow the normalization of CVR results to the standard MNI space. Next, the server will perform the CVR processing automatically. Specifically, 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-CO2 change (%BOLD/mmHg CO2) will be obtained. The primary outcome variable of the CVR measure is the gray matter CVR value, with lobar CVR as secondary variables. Once completed, the users will download a set of output results from the server, including the CVR maps and CVR values of the whole brain gray matter and different anatomic brain regions.

References

1. Liu P, Lin Z, Hazel K, Pottanat G, Xu C, Jiang D, et al. Cerebrovascular reactivity (CVR) MRI as a biomarker for small vessel disease related cognitive decline: validation in the MarkVCID Consortium. Proc Intl Soc Mag Reson Med; Toronto, CA2023. p. 408.
2. Liu P, Jiang D, Albert M, Bauer CE, Caprihan A, Gold BT, et al. Multi-vendor and multisite evaluation of cerebrovascular reactivity mapping using hypercapnia challenge. Neuroimage. 2021;245:118754.

3. Lu H, Kashani AH, Arfanakis K, Caprihan A, DeCarli C, Gold BT, et al. MarkVCID cerebral small vessel consortium: II. Neuroimaging protocols. Alzheimers Dement. 2021;17(4):716-25.
4. Liu P, Baker Z, Li Y, Li Y, Xu J, Park DC, et al. CVR-MRICloud: An online processing tool for CO2-inhalation and resting-state cerebrovascular reactivity (CVR) MRI data. PLoS One. 2022;17(9):e0274220.