MarkVCID MRI Free Water Imaging Biomarker Kit Protocol

I. Executive Summary

A Free Water (FW) kit is being proposed for the Susceptibility/Risk category. A secondary aim is to demonstrate that FW can also be used for Prognosis after sufficient longitudinal data is available. Free water content has been recently identified as a very sensitive and early biomarker of WM injury in association with vascular risk factors[1] and cognitive impairment[2-4] (see Kit Rationale Section) and, while WMH development is one of the last pathophysiological manifestations of the WM degeneration cascade and is likely to reflect permanent damage,[5-7] it is unknown whether FW content might be potentially modifiable. Therefore, we propose the **mean white matter free water fraction** (mFW) as an imaging biological marker candidate to *support early identification of VCID subjects at risk for cognitive decline.*

Cognitive Outcome Predicted by mFW

The main cognitive outcome predicted by mFW will be an executive function composite (EFC) score as assessed by an item-response theory generated score[8, 9] based on four executive tasks, performed as part of the MarkVCID Neuropsychological Testing Battery.

Hypotheses

For this study, we propose testing the following hypotheses:

Primary prespecified hypothesis (Susceptibility/Risk): Baseline mFW will be associated with baseline EFC measures. It is expected that larger baseline mFW will be associated with lower baseline EFC score.

Secondary hypothesis (Prognosis): Baseline mFW will be associated with the rate of change in EFC score. It is expected that increased mFW at baseline will predict accelerated EFC decline over time.

Timeline for validation

	Year 1	Year 2	Year 3
Disseminate computing algorithm to sites	x		
Site training on use of the algorithm	x		
Intra- and inter-rater reliability measures	x		
• Disseminate dummy, coded image sets to each site	x		
• Collect data from all sites	x		
• Analyze to evaluate reliability	x		
Inter-site variability estimates	x		
• Download Inter-site Variability Study data acquired by the MarkVCID Coordinating Center	x		
• Analyze Inter-site Variability data and calculate ICC	x		
• Present results to Imaging Biomarker subcommittee and discuss any "correction factors" that need be applied.	x		
Determine association between baseline mFW and baseline EFC score		x	
Determine association between baseline mFW and longitudinal trajectory of EFC score			x

II. Kit overview

Category

We propose mean white matter Free Water fraction (mFW) as a Neuroimaging Biomarker for Susceptibility/Risk. A secondary application based on longitudinal analysis will validate the kit for Prognosis.

Kit components, IP considerations

This kit will contain five files:

- Readme.pdf: this file contains the prerequisites and instructions to use the mFW algorithm
- MAIN_script_FW.sh: script that launches the different steps to generate the final mFW metric
- mrn.py: script that computes the mFW image
- FMRIB58_FA_1mm.nii.gz: FSL FA template
- FMRIB58_FA_1mm_thr.nii.gz: White matter mask image in FSL DTI template space

The proposed mFW algorithm will be an open-source software package. It was developed by Dr. Arvind Caprihan (UNM/MRN) and closely reproduces the results of the approach proposed by Pasternak et al.[10].

Kit rationale

Recent studies using diffusion tensor imaging (DTI) suggest that WM degeneration is a continuous and insidious process[11, 12]. Importantly new biomarkers of subtle WM injury such as DTI-derived fractional anisotropy (FA) and mean diffusivity (MD) demonstrate associations between WM integrity and vascular risk factors, even in the younger adult population, decades before white matter hyperintensities (WMH) appear or cognition declines.[13, 14]. Briefly, DTI-derived FA reflects the average diffusion anisotropy in a voxel, which includes the restricted diffusion within the axons and is contaminated by the partial volume of the extracelluar water.[15] The extracellular water is not only limited to cerebrospinal fluid partial volume effects at the border of the ventricles and brain parenchyma, but also within deep WM structures[16], reducing the sensitivity and specificity of most metrics derived from DTI.[2]

Recently, a method to correct DTI data for extracellular water contamination has been proposed.[10] Resulting “extracted” excessive water, referred to as free water, is modeled by a tensor that is isotropic and reflects the amount of water molecules that are not restricted by their surroundings. A voxel-wise FW image, just like a FA and a MD image can be calculated. There is accumulating evidence that supports the use of a bi-tensor model for separating free water from WM tissue improves specificity of resulting FW-corrected DTI-derived metrics, including FA and MD,[17, 18] . A recent longitudinal multisite study of healthy elderly subjects also revealed a significant reduction in the test-retest reproducibility error of all FW-corrected DTI-derived metrics studied in most regions of interest, and consistently across MRI sites.[19]

FW images have received recent attention for their sensitivity to reflect cerebral injury in association with vascular risk factors in relatively healthy adult individuals[1] but also with cognition in clinical patients populations[2-4]. In a recent longitudinal DTI study, we found cross-sectional and longitudinal associations between mFW and trajectory of cognitive and functional performance, including episodic memory, executive functions and Clinical Dementia Rating (CDR) scores in a large sample of cognitively diverse individuals[20].

Preliminary data

In a sample of 536 cognitively diverse individuals from the ADC UCD cohort, aged 77±8 years who received yearly comprehensive clinical evaluations and a baseline MRI exam[20], mFW was computed within WM voxels of each individual using the same approach than proposed in this protocol. The outcome included scores from a composite measure of executive function (EF) created from a set of fluency and working memory measures. Baseline mFW metric was then used as independent variable to explain baseline and change in EF using linear regressions, adjusting for relevant nuisance variables. Higher baseline FW was found to be associated with lower EF (β=-0.29, $p<0.001$) and accelerated EF decline (β=-0.32, $p<0.001$, see Figure 1**Error! Reference source not found.**). These findings support the relevance of FW content as a promising VCID biomarker.

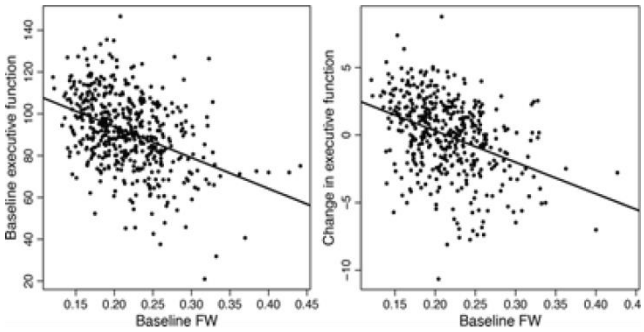

Figure 1: Association of baseline FW with baseline EF and rate of change in EF

III. Participating sites

Six MarkVCID sites have agreed to cross-validate the FW kit (UCSF/UCD/UCLA, JHU, UKY, USC, UNM, Rush), in addition to the availability of relevant legacy samples from CHARGE.

IV. Protocol for MRI acquisition

The MRI experiment protocol is to the same as that used for the PSMD kit, i.e.:

FOV (mm)	256
Number of Slices	80
TR (ms)	9800
TE (ms)	84
Base Resolution (voxels)	128
Voxel Size	2.0x2.0x2.0 mm^3
Phase Partial Fourier	(6/8)
PAT MODE	GRAPPA
Accel. Factor PE	2
IPAT Slice or MB	1
Echo Spacing (ms)	0.7
BW (Hz/pixel)	1628
Number of b ≠ 0 scans (gradient directions)	40
b0	0
Number of b = 0 scans	5
b1	1000
Phase Encoding Directionality Mode	AP and PA (2nd scan)
Distortion Correction Method	topup/eddy

V. Additional data collection required for analysis

To validate the utility of this method, we will require subject demographics, vascular risk factors along with the cognitive testing battery regularly acquired for the MarkVCID project. Table A1 in the Appendix section contains a list of the data required for the kit.

VI. Protocol for MRI analysis

Pre-processing (not part of the proposed kit) is the same as PSMD kit pre-processing

Briefly, pre-processing of DTI data can be performed using dcm2niix and FSL tools and includes the following steps:

- Conversion from DICOM to NIfTI files using dcm2niix
- Correction for Susceptibility-induced Distortions using the module TOPUP from the FSL package
- Correction for eddy current and using eddy from the FSL package
- Tensor fitting using dtifit from the FSL package

Using a standard desktop computer, pre-processing of DTI data takes approximatively 2 hours.

mFW processing (see Figure 2)

The following steps will be automatically performed through the provided FW script:

1. Compute FA map using dtifit (from FSL)
2. Estimate transformation parameters obtained by coregistrating the FA map to the FSL standard FA template
3. Compute the FW map
4. Coregister the FW map in the template space using transformation parameters from step 2
5. Compute the biomarker mFW (mean FW over the provided white matter mask)

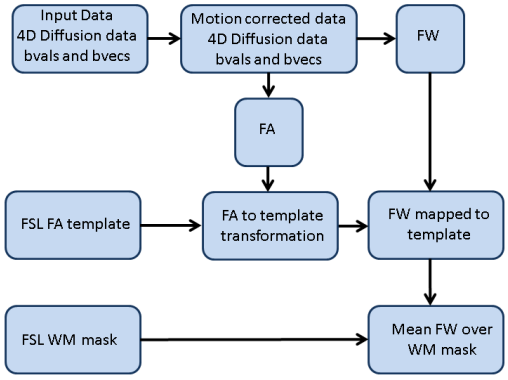

Figure 2: The Pipeline for mFW processing is shown.

Technical requirements

Hardware:

- Computer with Linux or Mac OS X.
- For Windows, a Linux virtual machine is needed, e.g. the NeuroDebian Virtual Machine (http://neuro.debian.net/vm.html)

Software (freely available tools):

- Mandatory:
  - An installation of the FMRIB Software Library (FSL, https://www.fmrib.ox.ac.uk/fsl).
  - An installation of Python 3.6.5 and of the following python libraries: “matplotlib” and “dipy”.
  - An installation of R cran (https://cran.r-project.org/)
- dcm2niix (https://github.com/rordenlab/dcm2niix). This tool is designed to convert neuroimaging data from the DICOM format to the NIfTI format. This software is open source. The bulk of the code is covered by the BSD license. Some units are either public domain or use the MIT license. Other DICOM-to-NIfTI convertor tools can be used if in addition to converting the DICOM files to a 4D NIfTI file, they also extract the b-values and b-vectors to separate files.

Instruction to run the program

The mFW algorithm will be run using a single command-line-based program that requires (1) 4D pre-processed DTI volumes, (2) bval file, (3) bvec file, (4) FSL FA template (provided), and (5) white matter mask image in FSL template space (provided). During processing, the algorithm will compute a FW content fraction map in the subject’s DTI-image space as well as in the FSL template space. It will finally generate the measure proposed by this kit: mFW, i.e. average of FW fraction values computed within voxels from the white matter compartment.

Technical specificity

After the data has been motion and distortion corrected by the PSMD kit scripts, mFW processing will take approximatively 4 minutes with a standard desktop computer. Instructions and scripts to launch the mFW pipeline are provided as a separate zip file “scripts_FW_CONSORTIUM.zip”. Prior to be launched for the first time, paths for programs’ directory have to be modified by the user in the main script (MAIN_script_FW.sh). Detailed instructions are provided in the Readme.pdf file.

Potential pitfalls

At the first use, users may encounter error message for the following reasons: first, one or more Python library may be missing. Users should contact their IT department to fix this issue if using a centerwide Python distribution or install the necessary packages if using a local Python installation. Second, paths for program’s directory, modified by the user in the main script before initial launch, may be incorrect. Users can contact Dr. Caprihan (acaprihan@mrn.org) or Dr. Maillard (pmaillard@ucdavis.edu) if they encounter any other issues.

VII. Step-by-step analytic plan

Instrumental validation

Sites which participate in the PSMD kit validation (UCSF/UCD/UCLA, JHU, UKY, USC, UNM, Rush, CHARGE) will use DTI images they collected for that kit in the instrumental validation process.

- **Inter-rater reliability:** Baseline brain MRI scans from three participants at each site, selected to represent a range of disease severity will be shared with other sites to be used in the characterization of inter-rater reliability. The lead sites (UCSF/UCD/UNM) will select 3 subjects from each site and make this data available to all sites through Globus. Each site will estimate FW measures on all the 21 subjects from the seven sites and share results with the lead site.

  Step-by-step procedure:

  1. Each site downloads inter-rater reliability dataset from MarkVCID Globus server
  2. Each site runs mFW script and calculates the mFW measure and calculates inter-rater reliability statistics for that site
  3. Each site shares mFW measures with the Lead Site
  4. The Lead Site computes inter-rater reliability statistics**.** We anticipate it will be close to 1 since FW estimation is entirely automated.

- **Test-retest reliability:** For instrumental validation at their own sites, each site will obtain DTI scans at baseline (t0) and one-week later (t1) for 6 participants and calculate ICC between FW measures computed from t0 and t1 DTI scans.

  Step-by-step procedure:

  1. Site receives MRI data from other sites
  2. Site runs FW script and obtains FW measures from t0 and t1 DTI scans
  3. Site calculates ICC
  4. Site reports results to Lead Site

Patient exclusion criteria for main analysis

Exclusion of participants with large artery infarcts or hemorrhages on MRI is recommended, as these may affect the estimation of mFW.

Definition of executive function composite (EFC) variable

The EFC score will be an item-response theory (IRT) generated score[8, 9] based on four executive tasks from the UDS 3.0 which are part of the MarkVCID Neuropsychological Testing Battery (https://markvcid.partners.org/filebrowser/download/1235) and collected by each site at each visit.

The four executive tasks include:

- Trails B (number of correct lines per minute)
- Number Span Test Backward (total score)
- Phonemic fluency (number of correct F-words in one minute)
- Category fluency (number of correct animal responses in one minute).

Procedure to compute EFC score:

IRT scores will be built using baseline UDS data from 3,450 clinically normal subjects from the NACC database. Dr. Adam Staffaroni from UCSF will use the NACC controls to derive the factor structure and loadings using the R *ltm* package[21]. The parameters from this analysis will be included in an R script, made available online on the MarkVCID portal. Each site will be able to automatically compute the EFC composite scores by providing, as an input for this script, a dataset containing the four executive tasks described above.

Procedure to compute rate of change in EFC score:

EFC score obtained at baseline will be subtracted from EFC score obtained at follow-up. This score difference will then be normalized by the time between baseline and follow-up neuropsychological exam dates.

mFW measurement

Each site will generate individual-level mFW from DTI data with the script provided with the kit (see kit components).

Statistical analyses

Cross-sectional analysis: Association between FW and baseline EFC score

Use linear regression with baseline EFC score as the dependent variable and baseline FW content as the independent variable, adjusting for baseline age, sex and education.

Model 1: EFC ~ mFW + age + sex + education

In a second model, additionally adjust model detailed above for diabetes, smoking and hypertension. This model aims to estimate the added contribution of FW above vascular risk factors on executive function performances.

Model 2: EFC ~ mFW + age + sex + education + diabetes + smoking + hypertension

Longitudinal analysis (optional): Association between mFW and rate of change in EFC score

Use linear regression with rate of change in EFC score (ΔEFC) as the dependent variable and baseline mFW content as the independent variable, adjusting for baseline EFC, age, sex and education.

In a second model, additionally adjust model detailed above for diabetes, smoking and hypertension.

Model 1: ΔEFC ~ mFW + EFC + age + sex + education

Model 2: ΔEFC ~ mFW + EFC + age + sex + education + diabetes + smoking + hypertension

- Scripts in R language can be provided upon request to Lead Site (please contact Dr. Maillard, pmaillard@ucdavis.edu) to perform all statistical analyses listed above.

VIII. Sample size calculation

Cross-sectional analysis: In the UCD ADC cohort, we used a composite measure of executive function, including the following tests: 1) Category fluency, 2) Verbal fluency and 3) Digit Span Backwards, and 4) Visual Span Backwards. The partial correlation between baseline FW and the composite score, adjusting for age, sex, and educational level, was ρ=-0.25. To detect a partial correlation of that size with 80% power requires a sample size of around n=123. Therefore, we recommend a minimum sample size of n=123 participants per site to be adequately powered to run this kit.

Longitudinal analysis (optional): Using the same sample, we found that the partial correlation between baseline FW and annual change in the composite score of executive function, adjusting for age, sex, and educational level, was ρ=-0.26. To detect a partial correlation of that size with 80% power requires a sample size of n=122.

Potential pitfalls: Cognition was measured using a slightly different set of measures [1) Category fluency, 2) Verbal fluency and 3) Digit Span Backwards, and 4) Visual Span Backwards] than those proposed for this MarkVCID protocol [1) Category fluency, 2) Verbal fluency, 3) Number Span backward and 4) Trail B measures] for our power estimate. This could influence power estimates.

IX. Plan for longitudinal data collection analysis

For the secondary hypothesis (optional), the analysis requires at least two clinical evaluations in order to compute the rate of change in global cognitive function. Three or more measures would afford the use of mixed models with random slope and intercept. This would substantially increase the power to detect associations with FW. The same analysis as above should be performed with the annual change in the composite score of executive function.

X. Plan for reporting outcomes

We fully expect that each site will share their data with the MarkVCID Coordinating Center to create the best opportunity to advance science. We believe this would allow us to analyze, present and publish manuscripts of our results. We predict that conferences such as the AAIC, AAN and VasCog would be ideal venues to present preliminary data. The results of our work could also entice pharmaceutical company interest in the treatment of VCID, resulting in new clinical trials.

The UCSF/UCD/UNM group will do all they can to support the acquisition, quality control and reporting of the results.

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

We plan to share all necessary FW content Kit components (program's package and manual) with participating sites. This and additional information (guide to perform proposed statistical analyses with R) will also be shared through a dedicated FW folder on the MarkVCID portal.

Biomarker and outcome data collected in CHARGE participating cohorts through previously funded projects will be shared with other UH2/UH3 investigators as mutually decided by the SVD Biomarker consortium. New data generated in the UH3 phase will also be shared with participating sites.

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Appendix

Table A1 Data required to run the kit

Variable	Description
ID	Participant ID
MRI date	Date of baseline MRI
Health status	Cognitively normal=0, MCI=1, Demented=2
Cognitive evaluation date	Date of cognitive evaluation visit date at baseline
Number Span Test Backward	Number of correct trials [0-14]
Category Fluency – Animals	Total number of animals named in 60 seconds [0-77]
Phonemic Fluency –Words beginning with F	Number of correct F-words generated in 1 minute [0-15]
Trail Making Test B	Total number of seconds to complete [0-300]
Age	Age of the participant at MRI
Sex	Sex
Education	Participant's educational level
Diabetes	Diabetes (yes/no)
Smoking	Current smoking (yes/no)
Hypertension	Hypertension (yes/no)