MarkVCID MRI White Matter Hyperintensity (WMH) Volume
Biomarker Kit Protocol

Background

White matter hyperintensities (WMH) are considered one of the paradigmatic markers of cerebrovascular disease. First identified in 1986 by Awad and colleagues[1], multiple cross-sectional studies[2], including population based studies[3] have found significant associations between age and cerebrovascular risk factors—particularly hypertension—and WMH. Prospective longitudinal studies also show associations between WMH and future risk of stroke, mild cognitive impairment, dementia and death[4]. Similarly, prospective studies of cognitively normal[5] and cohorts of mixed cognitive ability[6] find associations between both baseline and growth of WMH and cognitive decline.

Neuropathological studies find a variety of pathological substrates to WMH[7-10] mostly associated with vascular brain injury, although more recent studies also suggest that amyloid and tau pathologies also may be associated with WMH[11-14]. Anatomical localization of WMH, however, may be useful to differentiate these pathologies[15-17].

The substantially developed scientific literature on the subject of WMH has contributed to the formation of multiple guidelines related to use in the diagnosis of vascular cognitive impairment[18-21] as well as the development of Neuroimaging standards for research into small vessel disease[22].

Despite being one of the earlies identified and most studied of the MRI vascular markers, quantitative WMH measures have been used only infrequently in clinical trials[23], although a recent substudy of the SPRINT study (SPRINT-MIND) was recently reported to show significant reduction in the rate of WMH accrual with hypertension treatment.[1]

The development of a standardized, easily shareable approach to quantification of WMH with direct application to clinical trials aimed to reduce or prevent vascular brain injury, therefore, remains highly relevant. We hope that our imaging biological marker of WMH will suitably fulfill the criteria established by the MARK VCID consortium to serve in this role.

Executive Summary

We propose the Neuroimaging Biomarker of WMH. This biomarker is specific to alteration in water content of cerebral white matter. While this biomarker could be used as treatment outcome, more work is needed to assess the ability to measure change over a short period of time with this biomarker as well as the impact of therapy on this biomarker. Therefore, the most prudent use of this biomarker is as a stratification variable to enrich study populations for vascular disease.

[1] Nasrallah, I; A randomized trial of intensive versus standard systolic blood pressure control on brain structure: results from SPRINT MIND MRI. AAIC 2018 conference: 8/25/2018, Chicago, IL.

Hypotheses:

For this study, we propose testing the following hypotheses:

1) This measure will be highly reproducible across various MRI machines. It is expected that the coordinating center will test this hypothesis by having a small group of individuals imaged using a standard FLAIR sequence developed by the Imaging Subcommittee at each participating site.
2) This measure will associate with vascular risk factors. It is expected that individuals with long-standing hypertension will have greater WMH volumes than those with recent or no history of hypertension. Individual sites will be required to obtain life course history of blood pressure in order to be eligible to participate in this aspect of the study.
3) This measure will associate with the presence of MRI infarction. This has been proven in the past[24, 25] but this study would give us the opportunity to confirm the finding in a more standardized manner.
4) This measure will associate with cognition (executive function composite score generated from item response theory). In particular, WMH have been repeatedly shown to associate with measures of processing speed. Using standardized methods may further extend the utility of this measure in clinical trial design.
5) This measure will associate with serum biomarkers of endothelial injury and inflammation. Given the role of increased vascular resistance in white matter injury[26, 27], we hypothesize that WMH will reflect a state of endovascular injury and consequential inflammation.
6) This measure with increase over time in this "observational" cohort. Again, this has been repeatedly shown across a variety of cohorts[5, 6, 28, 29], but further testing of this measure in a cohort where vascular disease measures are deemed important will further validate the marker.

Proposed Etiology of WMH

The specific aspect of subcortical vascular disease pathology measured by WMH is uncertain. It is clearly apparent that there is attenuation of cerebral myelin and increase in white matter astrocytes from studies of postmortem pathology[9]. While it has been mostly argued that these changes result from ischemic injury[30], the picture is likely to be more complex[9, 10, 31, 32] suggesting break down of the blood brain barrier[33], inflammation and immune reaction. More recent studies by our laboratory also support the notion that WMH reflect the end-point of a cascade of subtle white matter injury[27, 34, 35] that may be the consequence of altered microvascular environment. This hypothesis served as the impetus for the UCD/UCLA/UCSF collaboration as part of the MARK VCID consortium.

Outcome Measured by the WMH

We believe that cognitive measures would be the best outcome for WMH. For example, individuals with extensive WMH at baseline would be significantly associated with decline in cognitive abilities related to processing speed (e.g. Trails B). Incident stroke, mild cognitive impairment or dementia could be other outcome measures, but this will require follow-up intervals of more than 2 years[4], making these outcomes less likely to achieve.

Timeline for validation

Year 1:

1) Disseminate computing algorithm to sites
2) Site training on use of the algorithm
3) Intra- and inter-rater reliability measures
    a. Disseminate dummy coded image sets to each site
    b. Collect data for all sites
    c. Analyze to evaluate reliability
    d. Address low reliability with further training
4) Inter-site variability estimates
    a. Work with coordinating center to acquire MRI on a few subjects across all sites
    b. Analyze data and calculate ICC
    c. Present results to imaging subcommittee and discuss any "correction factors" that need be applied.

Year 2:

5) Cross-sectional association of WMH with risk factors, cognition and serum biomarkers (if available)

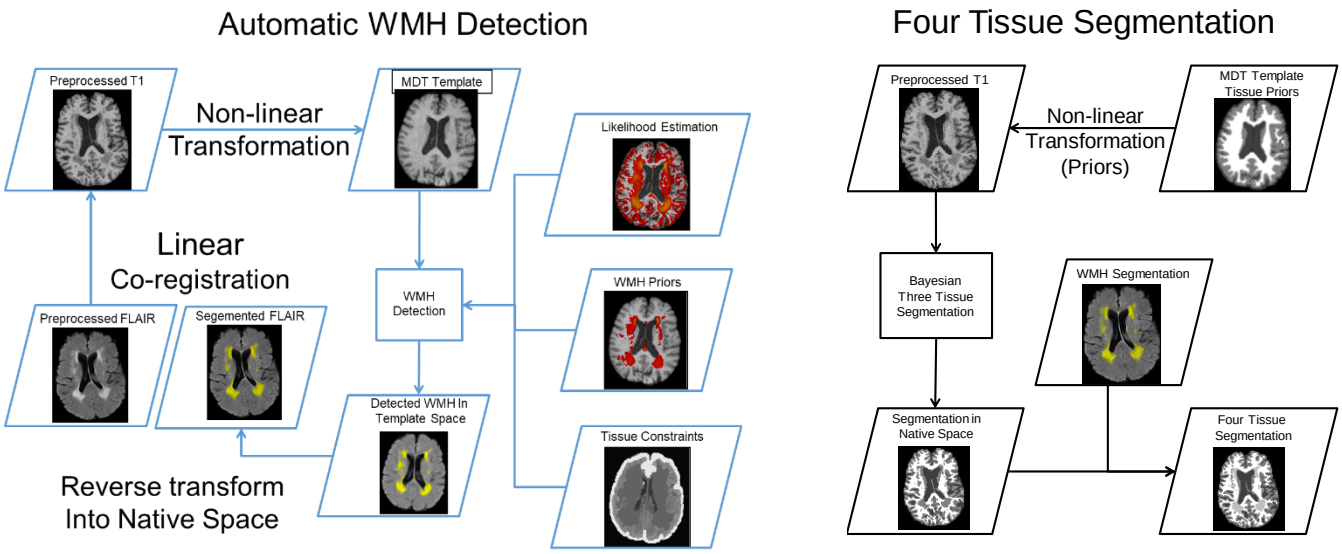
Year 3:

6) Application to longitudinal differences in cognition would take a minimum of 1 year, although 2 years would be substantially better.
7) Association of longitudinal differences in global WMH to vascular risk factors, cognition and serum biomarkers.

<u>Brief description of the Biomarker Kit</u>

This kit will be a command-line program that requires only 3 inputs: 1) High resolutions 3D T1 image, 2) a FLAIR image and 3) a brain mask from the 3DT T1 image. The algorithm will return a four level gray scale segmented image in native space of the 3D T1 that includes CSF, Gray Matter, White Matter and White Matter hyperintensities along with a segmented mask of WMH. The process is summarized in Figure 1.

Figure 1.



WMH volume from the output image can be calculated by the number of voxels identified as WMH multiplied by the voxel volume. If the consortium leaders were interested, the intermediate steps of the analysis could be saved to allow for voxel based analyses.

This program has very limited computing requirements and could be performed on most computers, including laptops. The currently available algorithm, however, requires a LINUX environment for successful application. No clinical data are required for normal algorithm performance.

Participating sites

All sites have chosen to participate. There should be no restriction to any site for this Kit.

Protocol for MRI acquisition

The neuroimaging subcommittee has decided on a 1mm isotropic 3D FLAIR imaging at 3 Tesla. The exact protocols will be machine dependent, but are available for all 3T platforms.

Additional data collection required for analysis

To validate the utility of this method, we will require subject demographics, baseline, life-time history of vascular risk factors and current vascular disease along with regularly acquired cognitive testing. Similarly, the blood biomarkers could be associated with these measures in cross-sectional and longitudinal fashion.

Protocol for MRI analysis

The analysis is summarized in Figure 1.
Specific steps require 3, 3D volumes to represent the raw 3DT1 image, the raw FLAIR image and the brain mask. These can be in Analyze or NIFTI format. Importantly, the algorithm assumes that the image orientation can be determined. We have found that NIFTI quaternion is the best representation of image orientation (see: https://nifti.nimh.nih.gov/nifti-

1/documentation/nifti1fields/nifti1fields_pages/quatern.html/view?searchterm=orientation for specifics).

The command line language:

UCD_WMH_Segmentation <3DT1 image.nii> <3DT1 brain mask.nii> <FLAIR.nii>

Step-by-step analytic plan

1) Linear co-registration of 3DT1 image to FLAIR image
    a. Includes scaling and orienting
2) Removal of non-brain elements from FLAIR image using 3DT1 brain mask
3) Image intensity normalization of FLAIR image
4) Non-linear warping of 3DT1 brain image to atlas image
5) Non-linear deformation of FLAIR using 3DT1 parameters
6) Application of Bayesian segmentation to 3DT1 and FLAIR
7) Create 4 tissue segmentation image
8) Reverse transformation of 3 tissue segmented image into 3DT1 native space
9) Reverse transformation of WMH segmented image into FLAIR native space
10) Reverse transformation of 4Tissue segmented image into 3DT1 native space
11) Write out these images into directory from which the program is launched.

Hypothesis Testing

Hypothesis #1: This measure will be highly reproducible.

Working with the coordinating center, each site will obtain repeated MRI measures of 6 individuals 1 week apart. We expect that each site of the Mark VCID consortium would participate in this process given their prior agreement with the coordinating center. The following measures will then be obtained from these data.

Intra-individual reliability. Each analyst at each site will be required to analyze the baseline images of the 6 individual MRIs twice. These images will be dummy coded so that the analyst will not be able to discern the repeated MRIs.

Inter-individual reliability. Results of the prior analysis will be compared across at least 2 raters at each site for the same images.

Machine and site dependent variability. We will utilize the harmonized imaging biomarker subcommittee imaging sequences. In collaboration with the Coordinating Center of Mark VCID, we will analyze site specific data obtained on a subset of same individuals.

Repeated Measures Reliability. Each site will be asked to repeat imaging on 6 individuals within 1 week to assess repeated measures reliability on their own systems.

Expected outcomes: The intraclass correlation coefficient (ICC) among raters for readings of baseline and change in WMH volume (a continuous endpoint). A ICC greater than 0.95 will be expected to qualify for acceptable reliability within and between analysts[36]. If test-retest intraclass correlation coefficient is 0.9, our power calculations will require 23 subjects (surpassed by 6 per site x at least 4 sites) to have 80% power to detect a difference from the

null hypothesis of ICC=0.7 and at least 30 subjects (6 per site x at least 5 sites) for 90% power. A similarly correlation would be expected for individuals images at 2 points close in time, yielding similar power.

Potential pitfalls: Lack of training on intracranial volume determination will be the greatest cause for error. If a site does not meet adequate reliability, the UCD/UCSF team will consult with the particular site to assure correct implementation of the protocol.

Hypothesis #2: This measure will associate with vascular risk factors. It is expected that individuals with long-standing hypertension will have greater WMH volumes than those with recent or no history of hypertension. Individual sites will be required to obtain life course history of blood pressure in order to be eligible to participate in this aspect of the study.

Expected Outcomes: We used linear regression analysis to estimate the association between the systolic blood pressure and WMH. To calculate power, Dr. Harvey used information from Table 3 in Scott et al.[11] to determine a sample size needed to detect an increase in R-squared of 0.0317 (square of the CC for blood pressure) using G-power, assuming alpha=0.05 and 80% power in cross-sectional analysis. This analysis resulted in an estimated sample size of 240 people.

Considering the means and standard deviations in Table 4 of the same paper, which compares mean WMH volume by normal/high blood pressure in both amyloid negative and amyloid positive individuals. If only amyloid positive people are included in the estimate and assuming equal numbers in the normal blood pressure and high blood pressure groups, only 70 people total (35 per group) would be needed to have 80% power to detect such a difference in means.

Potential pitfalls: Many sites will not have enough individuals in their cohorts to enable power to detect an effect. Three sites have identified enough subjects to show sufficient power, however.

Hypothesis #3 (exploratory, not part of kit validation)**.** This measure will associate with the presence of MRI infarction. This has been proven in the past[24, 25] but this study would give us the opportunity to confirm the finding in a more standardized manner.

Expected Outcomes: We used linear regression analysis to estimate the association between CVA and WMH volume. To estimate power, data from the Framingham Heart Study was used. These data were analyzed using the proposed WMH quantification method. Power analysis was performed using JMP PRO version 14. Assuming an alpha =0.05 and 80% power to detect a minimal significant group difference between those with and without stroke in cross-sectional analysis. This analysis resulted in an estimated sample size of 66 people.

Potential pitfalls: There is no “kit” for CVA assessment on MRI. This could lead to site-to-site variability in what is called an MRI infarct, reducing power to detect an association. We recommend use of the STRIVE criteria[22].

Hypothesis #4. This measure will associate with cognition. In particular, WMH have been repeatedly shown to associate with measures of processing speed. Using standardized methods may further extend the utility of this measure in clinical trial design.

Expected Outcomes: We used linear regression analysis to estimate the association between WMH volume and two measures of cognition adjusting for age, gender and educational achievement in years. Data from the UCD ADC diversity cohort was used. These data were also analyzed using the proposed WMH quantification method. Power analysis was performed using JMP PRO version 14. Assuming an alpha =0.05 and >80% power to detect a minimal significant association between WMH and episodic memory adjusted for age, gender and education. This analysis resulted in an estimated sample size of 104 people. The same analysis using executive function as an outcome resulted in an estimated sample size of 89 people.

For MarkVCID phase 2 we plan to examine similar associations between WMH and 2 explicit measures of cognition:

Primary cognitive outcome marker: item-response theory (IRT) generated z-score (trails B, backward digit span, phonemic fluency-F words, category fluency-animals)

Secondary cognitive outcome marker: Trails B

The global z-score and Trails B given our preliminary data shows significant associations with both memory and executive function in our ADC cohort. We expect that the sample size for these two measures will be similar to the numbers projected by our preliminary data.

Potential pitfalls: Cognition was measured using a different set of measures than those proposed for Mark VCID for our power estimate. This could result in inaccurate power estimates.

Hypothesis #5 (exploratory, not part of kit validation)**.** This measure will associate with serum biomarkers of endothelial injury and inflammation.

Expected Outcomes: We will use linear regression analysis to estimate the association between WMH volume and the proposed serum biomarker methods. Unfortunately, lack of substantial and reliable preliminary data limits our ability to accurately predict power at this time.

Potential pitfalls: The serum measures will prove to be either less sensitive or less reliable than expected resulting is weak or absent associations.

Hypothesis #6 (exploratory, part of longitudinal data collection but not part of kit validation)**.** This measure will increase over time in this "observational" cohort. This increase will be greater for those with hypertension.

Expected Outcomes: Using ADNI 2 data, we performed power estimates of longitudinal estimates of individuals with normal blood pressure as compared to those with high blood pressure leads to the following estimates:

25% reduction in increase in WMH: n=3129 per group

50% reduction in increase in WMH: n=782 per group

75% reduction in increase in WMH: n=348 per group

Final Note

These sample estimates are based on results using the same measurement algorithm as the proposed “Kit”, but were not done under the same controlled conditions of MRI acquisition as the proposed MARK VCID which will likely improve statistical power.

Plan for longitudinal data collection analysis

Analysis will depend somewhat on the proposed acquisition plan. As discussed above, images acquired less than 1 year apart are likely to be less informative. If the UH3 portion should extend beyond 1 year, then multiple yearly acquisitions would be possible. Analysis would depend on these conditions.

For example, if only 2 measures are available, then simple difference would suffice. A fully described statistical model using cognition as an outcome would include pertinent variables such as age, gender, head size, time between imaging and cognitive assessment. Natural log transformation of the WMH volumes will enable a more normal distribution as well. A fully described model of vascular risk factors would include age, gender, head size and individual measures such as blood pressure, glucose, cholesterol, symptomatic diseases such as MI, Stroke, renal or peripheral vascular diseases or a composite measure such as the Framingham Stroke Risk Profile[37].

Three or more measures would afford the use of mixed models with random slope and intercept. This would substantially increase the power to detect relevant associations with risk factors, other biomarkers or cognition.

Plan for reporting outcomes

We are cautiously optimistic that each site will share their data with the 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. I predict that conferences such as the AAIC, AAN, ANA 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 UCD/UCLA/UCSF group will do all they can to support the acquisition, quality control and reporting of the results.

Plan for sharing data, samples/images, protocols

We will make this “Kit” freely available to all who wish to use it through our website software download page: http://idealab.ucdavis.edu/software/. We will also be happy to share the source code with the coordinating center for whomever wishes to get this additional information. All images acquired under the VCID consortium agreement will be uploaded to the coordinating center servers along with any needed clinical data except for data that may affect PHI. We can share the imaging data in most formats, but prefer DICOM as this is HIPAA complaint.

References

1. Awad IA, Spetzler RF, Hodak JA, Awad CA, Carey R. Incidental subcortical lesions identified on magnetic resonance imaging in the elderly. I. Correlation with age and cerebrovascular risk factors. Stroke 1986;17:1084-1089.

2. DeCarli C, Murphy DG, Tranh M, et al. The effect of white matter hyperintensity volume on brain structure, cognitive performance, and cerebral metabolism of glucose in 51 healthy adults. Neurology 1995;45:2077-2084.

3. Jeerakathil T, Wolf PA, Beiser A, et al. Stroke risk profile predicts white matter hyperintensity volume: the Framingham Study. Stroke 2004;35:1857-1861.

4. Debette S, Beiser A, DeCarli C, et al. Association of MRI markers of vascular brain injury with incident stroke, mild cognitive impairment, dementia, and mortality: the Framingham Offspring Study. Stroke 2010;41:600-606.

5. Debette S, Seshadri S, Beiser A, et al. Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. Neurology 2011;77:461-468.

6. Maillard P, Carmichael O, Fletcher E, Reed B, Mungas D, DeCarli C. Coevolution of white matter hyperintensities and cognition in the elderly. Neurology 2012;79:442-448.

7. Awad IA, Johnson PC, Spetzler RF, Hodak JA. Incidental subcortical lesions identified on magnetic resonance imaging in the elderly. II. Postmortem pathological correlations. Stroke 1986;17:1090-1097.

8. Chimowitz MI, Estes ML, Furlan AJ, Awad IA. Further observations on the pathology of subcortical lesions identified on magnetic resonance imaging. Archives of Neurology 1992;49:747-752.

9. Simpson JE, Fernando MS, Clark L, et al. White matter lesions in an unselected cohort of the elderly: astrocytic, microglial and oligodendrocyte precursor cell responses. Neuropathol Appl Neurobiol 2007;33:410-419.

10. Simpson JE, Ince PG, Higham CE, et al. Microglial activation in white matter lesions and nonlesional white matter of ageing brains. Neuropathol Appl Neurobiol 2007;33:670-683.

11. Scott JA, Braskie MN, Tosun D, et al. Cerebral Amyloid and Hypertension are Independently Associated with White Matter Lesions in Elderly. Front Aging Neurosci 2015;7:221.

12. Scott JA, Braskie MN, Tosun D, et al. Cerebral amyloid is associated with greater white-matter hyperintensity accrual in cognitively normal older adults. Neurobiol Aging 2016;48:48-52.

13. McAleese KE, Walker L, Graham S, et al. Parietal white matter lesions in Alzheimer's disease are associated with cortical neurodegenerative pathology, but not with small vessel disease. Acta Neuropathol 2017.

14. McAleese KE, Walker L, Colloby SJ, et al. Cortical tau pathology: a major player in fibre-specific white matter reductions in Alzheimer's disease? Brain 2018.

15. Yoshita M, Fletcher E, Harvey D, et al. Extent and distribution of white matter hyperintensities in normal aging, MCI, and AD. Neurology 2006;67:2192-2198.

16. Lee DY, Fletcher E, Martinez O, et al. Regional pattern of white matter microstructural changes in normal aging, MCI, and AD. Neurology 2009;73:1722-1728.

17. Lee DY, Fletcher E, Martinez O, et al. Vascular and degenerative processes differentially affect regional interhemispheric connections in normal aging, mild cognitive impairment, and Alzheimer disease. Stroke 2010;41:1791-1797.

18. Román GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop [see comments]. Neurology 1993;43:250-260.

19. O'Brien JT, Erkinjuntti T, Reisberg B, et al. Vascular cognitive impairment. Lancet Neurol 2003;2:89-98.

20. Gorelick PB, Scuteri A, Black SE, et al. Vascular Contributions to Cognitive Impairment and Dementia: A Statement for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke 2011.

21. Hachinski V, Iadecola C, Petersen RC, et al. National Institute of Neurological Disorders and Stroke-Canadian Stroke Network vascular cognitive impairment harmonization standards. Stroke 2006;37:2220-2241.

22. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol 2013;12:822-838.

23. Dufouil C, Chalmers J, Coskun O, et al. Effects of blood pressure lowering on cerebral white matter hyperintensities in patients with stroke: the PROGRESS (Perindopril Protection Against Recurrent Stroke Study) Magnetic Resonance Imaging Substudy. Circulation 2005;112:1644-1650.

24. DeCarli C, Miller BL, Swan GE, et al. Predictors of brain morphology for the men of the NHLBI twin study. Stroke 1999;30:529-536.

25. Vermeer SE, Den Heijer T, Koudstaal PJ, Oudkerk M, Hofman A, Breteler MM. Incidence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke 2003;34:392-396.

26. Maillard P, Mitchell GF, Himali JJ, et al. Effects of Arterial Stiffness on Brain Integrity in Young Adults From the Framingham Heart Study. Stroke 2016;47:1030-1036.

27. Maillard P, Mitchell GF, Himali JJ, et al. Aortic Stiffness, Increased White Matter Free Water, and Altered Microstructural Integrity: A Continuum of Injury. Stroke 2017;48:1567-1573.

28. Carmichael O, Schwarz C, Drucker D, et al. Longitudinal Changes in White Matter Disease and Cognition in the First Year of the Alzheimers Disease Neuroimaging Initiative. . Arch Neruol 2010;In Press.

29. Gavett BE, Fletcher E, Harvey D, et al. Ethnoracial differences in brain structure change and cognitive change. Neuropsychology 2018.

30. Fernando MS, Simpson JE, Matthews F, et al. White matter lesions in an unselected cohort of the elderly: molecular pathology suggests origin from chronic hypoperfusion injury. Stroke 2006;37:1391-1398.

31. Simpson JE, Hosny O, Wharton SB, et al. Microarray RNA expression analysis of cerebral white matter lesions reveals changes in multiple functional pathways. Stroke 2009;40:369-375.

32. Xu H, Stamova B, Jickling G, et al. Distinctive RNA Expression Profiles in Blood Associated With White Matter Hyperintensities in Brain. Stroke 2010.

33. Huisa BN, Caprihan A, Thompson J, Prestopnik J, Qualls CR, Rosenberg GA. Long-Term Blood-Brain Barrier Permeability Changes in Binswanger Disease. Stroke 2015;46:2413-2418.

34. Maillard P, Fletcher E, Harvey D, et al. White matter hyperintensity penumbra. Stroke 2011;42:1917-1922.

35. Maillard P, Fletcher E, Lockhart SN, et al. White matter hyperintensities and their penumbra lie along a continuum of injury in the aging brain. Stroke 2014;45:1721-1726.

36. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med 2016;15:155-163.

37. Wolf PA, D'Agostino RB, Belanger AJ, Kannel WB. Probability of stroke: a risk profile from the Framingham Study. Stroke; a journal of cerebral circulation 1991;22:312-318.