Journal of Visualized Experiments

Evaluation of Cognitive Performance of Hypertensive Patients with Silent Cerebrovascular Lesions

--Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE61017R1
Full Title:	Evaluation of Cognitive Performance of Hypertensive Patients with Silent Cerebrovascular Lesions
Section/Category:	JoVE Behavior
Keywords:	Cerebral microbleeds, Neuropsychological assessment, Silent lacunes, Vascular cognitive impairment, White matter hyperintensities
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Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
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TITLE:

Evaluation of the Cognitive Performance of Hypertensive Patients with Silent Cerebrovascular Lesions

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KEYWORDS:

Cerebral microbleeds, Neuropsychological assessment, Periventricular hyperintensities, Silent lacunes, Vascular cognitive impairment, White matter hyperintensities

SUMMARY:

Here, we present a protocol to assess whether various types of silent cerebrovascular lesions are differentially associated with deficits in certain cognitive domains in a cohort of 398 hypertensive elderly Chinese, using a combination of neuropsychological tests and multi-sequence 3T MRI scanning.

ABSTRACT:

Evidence accumulated from the last decade has proven that silent cerebrovascular lesions (SCLs) and their underlying pathogenic processes contribute to cognitive decline in the elderly. However, the distinct effects of each type of the lesions on cognitive performance remain unclear. Moreover, research data from Chinese elderly with SCLs is scarce. In this study, 398 otherwise healthy hypertensive elderly subjects (median age 72 years) were included and assessed. All participates were required to complete a battery of structured neuropsychological assessment, including forward and backward digit span tests, symbol digit modalities test, Stroop test, verbal fluency test and Montreal Cognitive Assessment. These tests were used to assess attention, executive function, information processing speed, language, memory and

visuospatial function. A multi-sequence 3T MRI scanning was arranged within one month of the neuropsychological assessment to evaluate the burden of SCLs. SCLs were rated visually. Cerebral microbleeds (CMBs) and silent lacunes (SLs) were identified as strictly lobar CMBs and SLs or deep CMBs and SLs according to their locations, respectively. Similarly, white matter hyperintensities (WMHs) were separated into periventricular WMHs (PVHs) and deep WMHs (DWMHs). A series of linear regression models were used to assess the correlation between each type of SCLs and individual cognitive function domain. The results showed that CMBs tend to impair language-related cognition. Deep SLs affect executive function, but this association disappeared after controlling for other types of SCLs. PVHs, rather than DWMHs, are associated with cognitive decline, especially in executive function and processing speed. It is concluded that different aspects of SCLs have differential impact on cognitive performance in hypertensive elderly Chinese.

INTRODUCTION:

Silent lacunes (SLs), cerebral microbleeds (CMBs) and white matter hyperintensities (WMHs) are referred to as silent cerebrovascular lesions (SCLs). Two types of WMHs are recognized: periventricular WMHs (PVHs) and deep WMHs (DWMHs). SCLs were once regarded as benign lesions without clinical significance. After decades of research, SCLs are now confirmed to be linked to varying functional impairment and cognitive deficits[1,2]. Nevertheless, consistent evidence is still limited in the spectrum and magnitude of cognitive effects of different types of SCLs. Moreover, the underlying mechanisms are elusive.

Most previous studies either recruited hospital patients with severe medical conditions[3-5] or included participants with advanced cerebral small vessel diseases[6,7]. The heterogeneity of the participants among different studies has partly contributed to the inconsistent results. To exclude these confounding factors, we conducted the current one-centered research study as an attempt to provide a clear picture through assessment of a relatively large, pure cohort recruited from a primary care setting. Furthermore, previous studies have predominantly focused on one or two types of SCLs and did not fully evaluate the independent associations between individual SCLs and specific cognitive functions. Therefore, we assessed various types of SCLs in the current study.

Neuropsychological tests are widely used to assess cognitive function of specific domains. They are useful in differentiation between normal aging and early cognitive impairment. Results of properly conducted neuropsychological assessment are sensitive in discerning behavioral and functional deficits. A battery of structured neuropsychological tests was chosen, including forward and backward digit span tests, symbol digit modalities test (SDMT), Stroop test, verbal fluency test and Montreal Cognitive Assessment (MoCA). Scores from these tests were grouped and combined to represent performance in different cognitive domains[8,9]. Such a method is widely used and is time efficient. A major drawback is that different neuropsychological tests may partly overlap in their tested domains. A more specific alternative is to use computer-based assessment with well-designed modules constructed using the E-Prime system, which is time-consuming and may not be suitable for screening purposes.

In conclusion, we aimed to assess the associations between the burden of different SCLs and impairment of various cognitive domains. Furthermore, vascular risk factors and other types of SCLs were controlled for to determine the distinct and independent profiles of cognitive impairment of each type of SCLs.

PROTOCOL:

The study protocol was approved by the Institutional Review Board of the University of Hong Kong / Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB) for human research.

1. Participants

1.1. Recruit otherwise healthy elderly Chinese subjects (from 65 to 99 years old, mean age 72) with a history of hypertension for at least 5 years.

1.2. Exclude participants with any disease affecting the cognitive function and/or with any disability hindering the completion of the required assessment, including but not limited to stroke, dementia, encephalitis, depression, diabetes mellitus and coronary heart diseases.

1.3. Inform the participant the scope of the study before obtaining a written consent.

2. Neuropsychological assessment

2.1. Arrange an interview for each participant to administer a battery of neuropsychological tests focusing on six cognitive domains (**Table 1**) and to collect the demographic and clinical data. Review the participant's medical records to ensure the reliability of relevant information.

2.2. Forward/Backward digit span tests

2.2.1. Prepare groups of random digit sequences of increasing length (**Figure 1A**). Start with a three-digit sequence. Read out the digit sequence aloud at a rate of one digit per second. Ask the participant to immediately recall the digit sequence verbally in the Forward digit span test[14].

2.2.2. Have the participant to recall progressively longer digit sequences with one more digit each time the participant has successfully recalled the digit sequence without any error.

2.2.3. Give a different digit sequence of the same length if the participant has failed in the first trial of a specific length. End the test if the participant has failed again. Discontinue the test also when the participant has failed up to three times in total.

2.2.4. Record the longest length of the digit sequence the participant has successfully recalled without any error.

2.2.5. Start with a three-digit sequence and ask the participant to recall the digit sequence in a reverse order in the Backward digit span test. Follow the steps of Forward digit span test otherwise.

2.3. MoCA

2.3.1. Administer MoCA using the validated version. Use the Cantonese version to measure global cognitive function in our protocol and to construct compound domain scores [15,16].

2.3.2. MoCA verbal learning task: Read five words from different categories (“面孔”、“絲絨”、“教堂”、“雛菊”和“紅色” as Chinese characters for face, cloth, church, daisy and red color in our protocol, respectively) to the participant. Ask the participant to immediately recall the words. Repeat the reading and immediate recall a second time. Remind the participant about a delayed recall 5 minutes later. Assign one point to each correct word during the delayed recall.

2.3.3. MoCA naming task: Show pictures of three animals (lion, rhinoceros and camel in our protocol) and ask participant to tell their names. Assign one point to each correct name.

2.3.4. MoCA repetition task: Read a simple sentence to the participant and ask the participant to immediately repeat it. Repeat the procedure with a more complex sentence. Assign one point to each correct repetition.

2.3.5. MoCA drawing a cube task: Ask the participant to copy a cube printed on a sheet of paper in nearby blank space. Assign one point if the cube is copied correctly.

2.3.6. MoCA drawing a clock task: Ask the participant to draw a clock face with time at 11:10. Assign one point each for an accurate completion of the clock face, numbers and pointers, respectively.

2.4. Stroop test

2.4.1. Use the Chinese Translated Victoria Version of the Stroop test in our protocol[17].

2.4.2. Inform the participant to finish three sessions each with 24 stimuli printed in four different colors in 6 rows within a sheet of paper (**Figure 1B**). Start with dots (color naming), next with four Chinese characters (of meaning not related to any color; neutral color), and finally with four Chinese characters (of meaning related to a color but in another color different from their meaning, e.g., “紅” as a Chinese character for “red” printed in green; interference). Remind the participant to name the color of the printed stimuli (i.e., green, blue, yellow or red) and disregard their meaning.

2.4.3. Allow the participant to use the first 4 stimuli in each session as a practice to ensure a full understanding of the rules. Point out any error during the practice stage and encourage the participant to correctly name the color.

2.4.4. Remind and encourage the participant to complete the remaining 20 stimuli as quickly and accurately as possible. Record the time used by the participant to complete each session (excluding the practice stage).

2.5. SDMT

2.5.1. Pair 1 to 9 digits in the numeric order with nine unassociated symbols[18].

2.5.2. Print a list of the nine symbols in a random order without the corresponding digits (**Figure 1C**). Ask the participant to fill in the blank with the correctly paired digit below each symbol. Allow the participant to check back and forth the printed pairs for reference at any time of the test.

2.5.3. Allow the participant to try filling the first 10 blanks as a practice to ensure a full understanding of the rules. Point out any error during the practice stage and encourage the participant to be correct.

2.5.4. Remind and encourage the participant to fill in the blank as quickly and accurately as possible in the next 90 seconds. Record the number of correct responses in the written-SDMT.

2.5.5. Continue the test but ask the participant to provide the correctly paired digit verbally. Record the number of correct responses in the oral-SDMT.

2.6. Verbal fluency

2.6.1. Ask the participant to provide a verbal list of names belonged to each of the three categories (i.e. animals, vegetables and fruits) separately in one minute for each category[19].

2.6.2. Record the total number of names for each category.

3. MRI acquisition and Visual rating of SCLs on MRI

3.1. Perform a multi-sequence 3-Tesla MRI scanning for the participant using the parameters and including the sequences summarized in **Table 2**. Complete the MRI scanning within one month of the neuropsychological assessment.

3.2. Identify and visually rate SCLs on MRI according to standard criteria by experienced raters in an anonymous manner. Ensure good intra- and inter-rater reliability.

3.3. Use T1-weighted and fluid-attenuated inversion recovery (FLAIR) images to identify SLs (as hypointense foci of 2-15 mm diameter on both sequences) and their locations (**Figure 2A**). Re-confirm the SLs on T2-weighted images (as hyperintense foci at the same locations).

3.3.1. Search all brain regions in a pre-specified order from anterior to posterior and from one side to the other to avoid any omission (i.e. starting from frontal lobe, island lobe, basal ganglion, thalamus, temporal lobe, parietal lobe, occipital lobe, cerebellum and finally to brain stem, and starting from the left side and then to the right side).

3.4. Use susceptibility-weighted imaging (SWI) to identify CMBs (as punctuate or small round/oval hypointense foci of 2-10 mm diameter) and their locations (**Figure 2B**). Divide the whole brain region into 7 anatomical locations (i.e., cortex and grey-white junction, subcortical white matter, basal ganglia grey matter, internal and external capsule, thalamus, brain stem and cerebellum) according to the Brain Observer MicroBleed Scale (BOMBS)[10].

3.5. Label SLs and CMBs as strictly lobar SLs and CMBs, respectively, when they are confined to the lobar white matter. Label them as deep SLs and CMBs, respectively, when deep or infratentorial lesions are observed with and without additional lobar lesions[11,12].

3.6. Use T2-weighted and FLAIR images to identify WMHs (as bilateral, almost symmetrical hyperintense areas) (Figure 2C). Re-confirm WMHs on T1-weighted images (as isointense or hypointense areas at the same locations). Recognize PVHs and DWMHs separately. Use the Fazekas scale to rate the severity of WMHs[13].

3.7. Rate PVHs appearing as “caps” or pencil-thin lining, smooth “halo” and irregular signal extending into the deep white matter as grade 1, 2 and 3, respectively. Rate DWMHs appearing as punctate foci, small confluent areas and large confluent areas as grade 1, 2 and 3, respectively.

4. Statistical analysis

4.1. Perform all analyses using the statistical package SPSS 22.0 for MacBook.

4.2. Transform the participant’s score for each test using z transformation:

$$z\ scores = \frac{individual\ test\ score\ minus\ mean\ test\ score}{standard\ deviation}$$

4.3. Invert the Stroop test scores so that a higher score represents better performance.

4.4. Calculate a compound score for each cognitive domain by averaging the mean z score of all component tests under the same domain[8,9]:

The compound score for executive function = (z score of backward digit span + z score of Stroop interference + z score of verbal fluency) / 3

4.5. Use linear regression models to explore the association between each type of SCLs and cognitive function, adjusting for age, sex, and educational level. Perform further analyses after adjusting for vascular risk factors if significant associations are identified.

4.6. Conduct additional analyses after further adjustment for the other types of SCLs in order to assess the independency of the association between the load of a specific type of SCLs and cognition.

REPRESENTATIVE RESULTS:

The mean age of the 398 participants was 72.0 (from 65 to 99, SD = 5.1) years, and there were 213 men (53. 5%; **Table 3**). **Table 4** summarizes the neuropsychological assessment results. Only 5 participants had all four types of SCLs. One or more types of SCLs were found in 169 (42.5%) participants, and 35 (8.8%) and 17 (4.3%) participants had 2 and 3 types of SCLs, respectively (**Table 5**).

The degree of PVHs and DWMHs were separately examined for their associations with performance in different cognitive domains. The data confirmed an independent association between the burden of PVHs and worse performance in executive function and information processing speed (**Table 6**). An increasing load of CMBs was associated with impaired language-related performance. Additional adjustment for vascular risk factors and other types of SCLs did not affect the independent impact of CMBs on language function (**Table 6**). Although there was a significant association between the presence of SLs and worse performance on executive function, this association was lost following additional correction for other types of SCLs (**Table 6**).

FIGURE AND TABLE LEGENDS:

Figure 1: Test sheets for the neuropsychological assessment. (**A**) Forward digit span test. (**B**) Stroop test. (**C**) Symbol digit modalities test.

Figure 2: MRI Images of different kinds of silent cerebrovascular lesions. (**A**) Fazekas grade 2 PVHs and DWMHs on a FLAIR image. (**B**) A CMB on SWI. (**C**) A SL on T1-weighted image magnified on both T1-weighted and T2-weighted imaging. CMB, cerebral microbleed; DWMHs, deep white matter hyperintensities; PVHs, periventricular hyperintensities; SL, silent lacune.

Table 1: Neuropsychological tests of six different cognitive domains. MoCA, Montreal cognitive assessment. Original source: Reference[20].

Table 2: MRI sequences and main parameters.

Table 3: Demographic characteristics and vascular risk factors of 398 participants. BMI, body mass index; DBP, diastolic blood pressure; IQR, interquartile range; SBP, systolic blood pressure; SD, standard deviation. Original source: Reference[20].

Table 4: Neuropsychological assessment results. MoCA, Montreal cognitive assessment. Original source: Reference[20].

Table 5: Prevalence and distribution of different types of SCLs.
CMBs, cerebral microbleeds; DWMHs, deep white matter hyperintensities; PVHs, periventricular hyperintensities; SCLs, silent cerebrovascular lesions; SLs, silent lacunes. Original source: Reference[20].

Table 6: Association between severity of PVHs, presence of deep SLs or strictly lobar CMBs and the Z score of selected cognitive domains.
B, unstandardized beta coefficient; β, standardized beta coefficient; CMBs, cerebral microbleeds; NA, not applicable; PVHs, periventricular hyperintensities; SCLs, silent cerebrovascular lesions; SLs, silent lacunes; SE, standard error.
1, single variable linear regression models controlled for age, gender, educational levels and vascular risk factors (body mass index, hyperlipidemia, impaired glucose tolerance, smoking, drinking, systolic blood pressure and diastolic blood pressure); 2, multiple variables linear regression models controlled for age, gender, educational levels and the other two types of SCLs. *, $p < 0.05$. Original source: Reference[20].

DISCUSSION:
In the study, we have combined the results of a battery of neuropsychological assessment and findings of a multi-sequence MRI examination to evaluate the impact of different types of SCLs on various cognitive functions. The major types of SCLs were examined (i.e., CMBs, SLs and WMHs). As previous studies have revealed that SCLs in different locations may represent different pathology and lead to different consequences, we categorized CMBs and SLs into strictly lobar (i.e., lobar only without deep ones) and deep ones (with or without lobar ones), and separated WMHs into PVHs and DWMHs. A battery of structured neuropsychological tests was chosen to provide a comprehensive assessment of cognitive functions covering six domains (i.e., attention, executive function, information processing speed, language, memory and visuospatial function). Compound scores for each domain were constructed for statistical analyses.

PVHs adversely affect executive function and information processing speed. Strictly lobar CMBs are linked to impaired language dysfunction. SLs are associated with impaired executive function. We additionally controlled for the vascular risk factors and other types of SCLs to determine the independent effects of each type of SCLs on cognitive functions. All the above-mentioned associations are independent of the vascular risk factors except that the association between SLs and executive function has disappeared when controlled for PVHs; other associations are not affected by controlling for other types of SCLs. In conclusion, the protocol has successfully confirmed that the type of SCLs could differentially affect the cognitive

performance in different domains. In other words, different types of SCLs are associated with distinct profiles of cognitive impairments. As previous studies have observed clinical differences between patients with hypertensive and non-hypertensive ischemic stroke[21], results of the present study are relevant to patients with hypertension.

Other limitations of the current research must be noted. First, the incidence and number of lesions in individual participant are relatively low despite choosing a cohort of hypertensive elderly who should have a higher incidence of SCLs than healthy non-hypertensive elderly. A possible explanation is the exclusion of the participant with significant diseases such as dementia and other overt cardiovascular diseases. Such exclusion criteria have omitted the participant at an advanced stage of SCLs and therefore could have underestimated the burden and impact of the SCLs. Another explanation is that the burden of SCLs may be lower in Asians than Caucasians. In any case, a lower burden of SCLs in the cohort has hindered the opportunity in further exploration of the impact of individual type of SCLs and their strategic locations. The chosen battery of neuropsychological assessment has led to another limitation. Some of these tests have inherent overlaps in their evaluated domains, whilst others have been used in different protocols to assess different domains. These could have contributed to the inconsistencies between the present and published results. We have adopted neuropsychological tests that were most frequently used in the literature for specific cognitive domains. Modules using computer-based tests or functional neuroimaging studies developed for different individual domains should be used in future studies. Focal cerebral atrophy is a potentially important type of SCLs relevant to both hypertension and cognitive functions[22], warranting further studies.

It is crucial to ensure that the participant knows precisely what is required to do when a start signal is given during the neuropsychological assessment. A practice stage is generally available before the formal test, during which the participant's errors are pointed out for corrections. A unified standard should be adopted for different tests in all participants, and this was achieved by having the same person (M. ZHANG) to administer all the neuropsychological tests. The standard assessment procedures were reviewed every three months to ensure the uniformity.

ACKNOWLEDGMENTS:

This work was supported by matching and donation funds (Cerebrovascular Research Fund, SHAC Matching Grant, UGC Matching Grant, and Dr. William Mong Research Fund in Neurology awarded to Professor R.T.F. Cheung).

DISCLOSURES:

The authors have no conflict of interest to declare.

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Figure

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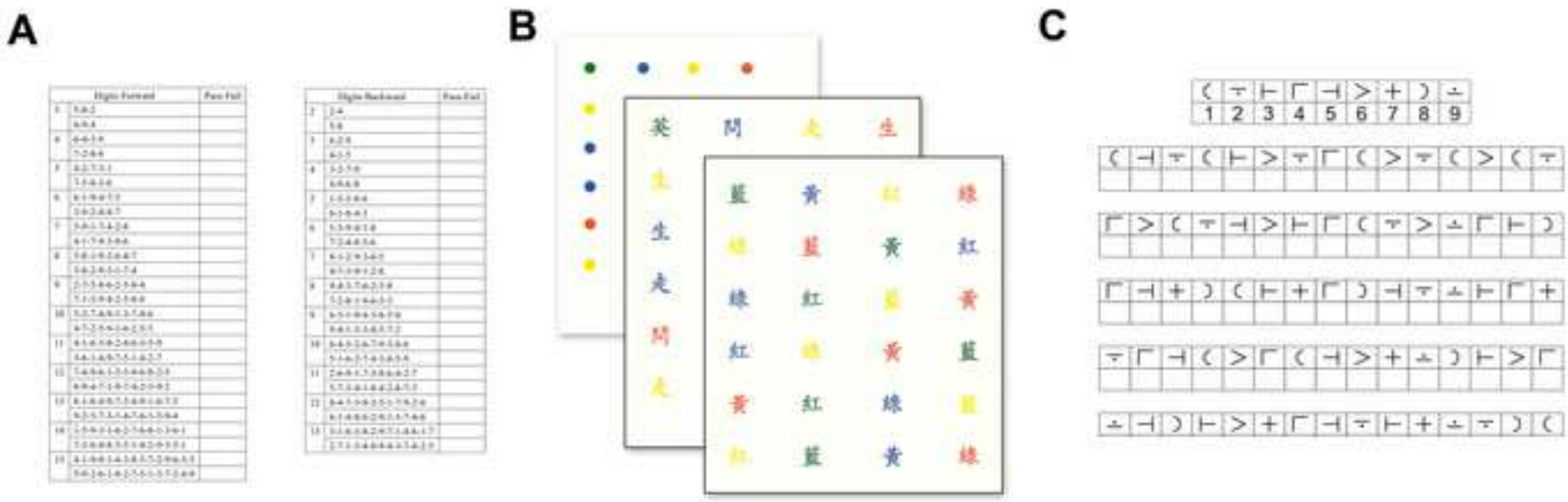

Figure

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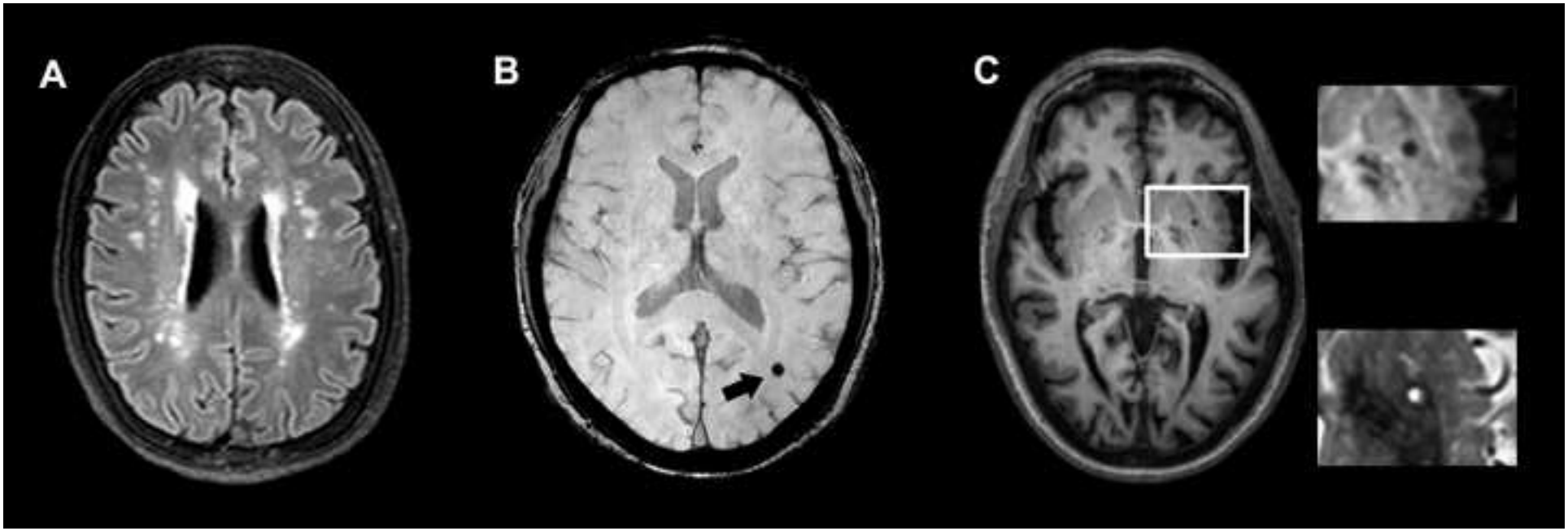

Cognitive domains
Attention
Executive function
Information processing speed
Language-related function
Memory
Visuospatial function

Neuropsychological tests
forward digit span, backward digit span
backward digit span, Stroop interference subtask, verbal fluency
Stroop color naming subtask, Stroop neutral color subtask, symbol digit modalities oral test, symbol digit modalities written test
MoCA naming, MoCA repetition, verbal fluency
MoCA verbal learning test
MoCA drawing a clock, MoCA copying a cube

MRI sequences	Repetition time	Echo time
Axial three dimensional T1-weighted magnetization prepared rapid gradient echo	7000 ms	3.2 ms
Axial proton density/T2 turbo spin echo run twice	5000 ms	16/80 ms
Fluid attenuated inversion recovery sequence	11000 ms	120 ms
Susceptibility weighted imaging	27.9 ms	23 ms

Inversion time	Slices	Slice thickness	Acquisition matrix size
/	155	1 mm	240 x 240
/	50	2.5 mm	480 x 480
2800 ms	50	1 mm	768 x 768
/	135	2 mm	704 x 704

Demographic characteristics	Number of participants
Male (%)	213 (53.5)
Mean age in years (SD)	72.0 (5.1)
Mean SBP in mmHg (% on drugs)	
<120	21 (5.3)
120-139	302 (75.8)
≥140	75 (18.9)
Mean DBP in mmHg (% on drugs)	
<80	265 (66.6)
80-89	114 (28.7)
≥90	19 (4.7)
History of smoking status (%)	84 (20.0)
History of heavy alcohol consuming (%)	14 (3.5)
BMI distribution (%)	
<25	228 (57.3)
25-29.9	146 (36.7)
≥30	24 (6.0)
Median educational level in years (IQR)	8 (6)

Neuropsychological tests	Mean score	Standard deviation
Backward digit span	4.6	1.6
Forward digit span	8	1.5
MoCA copying a cube and drawing a clock	3.4	0.9
MoCA naming	2.9	0.3
MoCA repetition	2.7	0.5
MoCA verbal learning test	12.5	2.4
Stroop color naming in s	18.7	5.9
Stroop neutral color in s	25.9	10.4
Stroop interference in s	43.1	17.5
Symbol digit modalities oral test	41.0	12.8
Symbol digit modalities written test	32.2	11.9
Verbal fluency	14.2	3.2

Types of SCLs	n (%)
PVHs	
Fazekas grade 1	176 (44.2)
Fazekas grade 2	191 (48.0)
Fazekas grade 3	31 (7.8)
DWMHs	
Fazekas grade 1	326 (81.9)
Fazekas grade 2	56 (14.1)
Fazekas grade 3	16 (4.0)
CMBs	
Strictly lobar	53 (13.3)
Deep	17 (4.3)
Both	15 (3.8)
SLs	
Strictly lobar	65 (14.8)
Deep	6 (1.50)
SCLs	
One type	112 (28.1)
Two types	35 (8.8)
Three types	17 (4.3)
All four types	5 (1.3)

	Executive function				Information processin	
	B	SE	β	*p* -value	B	SE
PVHs severity[1]	-0.143	0.059	-0.13	0.016*	-0.159	0.059
Strictly lobar CMBs[1]	NA				NA	
Deep SLs[1]	-0.235	0.012	-0.121	0.021*	NA	
PVHs severity[2]	-0.126	0.063	-0.106	0.046*	-0.149	0.064
Strictly lobar CMBs[2]	NA				NA	
Deep SLs[2]	-0.197	0.106	-0.098	0.064	NA	

g speed			Language-related function		
β	*p* -value	B	SE	β	*p* -value
-0.131	0.007*	-0.147	0.059	-0.128	0.014*
		-0.275	0.108	0.134	0.012*
		NA			
-0.116	0.020*	-0.107	0.062	-0.09	0.088
		-0.202	0.102	-0.098	0.049*
		NA			

Table of Materials

Name of Material/Equipment	Company	Catalog Number	Comments/Description
3T MRI	Philips Medical Systems		

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

REPLY

We are grateful for this kind reminder. We have thoroughly revised and proofread to ensure that there are no spelling or grammar issues.

2. Please format the manuscript as paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep, and note in the protocol section. Please use Calibri 12 points

REPLY

We have formatted the manuscript accordingly.

3. Please rephrase the Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

REPLY

We have rephrased the Summary accordingly.

4. Please ensure that the long Abstract is within 150-300-word limit and clearly states the goal of the protocol.

REPLY

The long Abstract contains 262 words. We have revised it to state the goal clearly.

5. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.”

We have revised the protocol section accordingly.

6. The Protocol should contain only action items that direct the reader to do something in a step by step manner.

REPLY
We have revised the Protocol accordingly.

7. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections.

REPLY
We have revised the Protocol accordingly.

8. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Please include discrete actions, button clicks in the software, the knob turns, etc.

REPLY
We have added more details to the protocol steps according to your advice.

9. 2.1: how do you perform multi-sequence 3T imaging? Please include all the button clicks and the knob turns if any?

REPLY
Table 2 describes the main parameters for performing the multi-sequence 3T MRI. See also 3.1.

10. 3.1: How was the assessment performed. Please include the details.

REPLY
We have added more details about the assessments performed in 2.1.

11. 3.2: How do you identify SLs? How would you look for hypointense foci on the scans?

REPLY

We have added more details about SLs identification in 3.3.

12. 3.3 How do you perform susceptibility-weighted imaging? What is the BOMB scale and how was it used for the assessment?

REPLY
Table 2 describes the parameters for SWI. We have added more details about the BOMBS and the identification of CMBs in 3.4.

13. 4.1. Please start this section with the patient entering the clinic, then providing informed consent to participate in the study, etc. Then provide examples of how to perform the trial. What is the length of numbers used in the first trial in your case?

REPLY
We have revised this section starting with the participant recruitment in 2.1 to 2.6. A figure (#1) is added to illustrate the test materials.

14. 4.2: Is this commercial? If yes, please use the generic term instead. If not, please include a citation. Please also include examples of words provided to the participants.

REPLY
A citation is added. We have included more details in 2.3.

15. 4.3: Is this commercial? If yes, please use the generic term instead. If not, please include a citation. Please include examples of 24 stimuli provided to the participant.

REPLY
A citation has been provided. We have included more details in 2.4 and added Figure 1B.

16. Same as above for all the tests.

REPLY
We have included more details for all the tests.

17. 5. For the analysis section, please include all the button clicks in the software used to perform the analysis.

REPLY
We have added more details in 4. However, we believe that describing button clicks in the software would make readers more difficult to understand the analyses.

18. There is a 10-page limit for the Protocol, but there is a 2.75-page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

REPLY
Filmable content is highlighted by a green background.

19. Please ensure that the Representative Results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. Data from both successful and sub-optimal experiments can be included.

REPLY
We have revised the Representative Results accordingly.

20. Please define all abbreviations during the first-time use.

REPLY
All abbreviations have been defined during the first-time use.

21. Please obtain explicit copyright permission to reuse any figures/table from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in Figure Legend, i.e. “This figure has been modified from [citation].”

REPLY
We have used new figure and new tables.

22. As we are a methods journal, please ensure that the Discussion to explicitly cover the

following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

REPLY

We have revised the Discussion accordingly.

Reviewers' comments:

Reviewer #1:

Major Concerns:

1. It would be interesting to know if there were a correlation between the number of silent lacunes and a higher degree of cognitive decline. In a recent clinical study more than half of patients with a first-ever lacunar stroke and without cognitive impairment showed minor neuropsychological alterations. These minor disturbances were mainly related to the presence of clinically silent lacunar infarcts, without any relationship to cognitive impairment with leukoaraiosis at this early stage of cerebral small vessel disease (see and comment the study published in BMC Neurology 2013; 13: 203)

REPLY

We agree with the reviewer. It would be interesting to assess the correlation between the number of silent lacunes and cognitive decline. However, the incidence and number of silent lacunes were lower than expected, making it not feasible to assess such correlation. We are interested to investigate this in future study. The independent cognitive disturbances of silent lacunes is an interesting topic, we have discussed it in our recent publication (J neuro sci 2019;403:139).

2. It would be helpful to mention that women differ from men in the distribution of risk factors and stroke subtype, stroke severity, and outcome (Clin Neurol Neurosurg 2014 Dec;127:19-24). It would be interesting to assess whether there were significant gender differences in the hypertensive patients analyzed in the present study.

REPLY

We are grateful for this excellent idea. We looked into our data but did not find any gender difference in this cohort.

Minor Concerns:

1. Authors should clearly state that the results of the study refer only to the group of patients with hypertension. This is relevant since a clinical study that analyzed differences between hypertensive and non-hypertensive ischemic stroke, reported that up to 25% of patients with lacunar infarctions had no hypertension. (Eur J Neurol 2004; 11: 687-692). A comment of this article can be added in the Introduction.

REPLY
We agree with the reviewer and have included this point in the discussion and added the reference.

2. Reword the title of the manuscript adding "hypertensive" to "patients"

REPLY
Thanks for the suggestion. We have reworded the title accordingly.

3. The presence and progression of cerebral atrophy is another potentially relevant silent manifestation (although still poorly characterized) of cerebral small vessel disease (Cerebrovasc Dis. 2010;30(2):157-66; Neurology. 2012 Nov 13;79(20):2016-7). The authors should mention that an essential line of future research would be precisely the assessment of cerebral atrophy and its potential relationship with cognitive impairment in silent hypertensive small vessel disease. Add and comment these references.

REPLY
We agree with the reviewer and have included this point in the discussion and added the reference.

Reviewer #2:
Major Concerns:
1. Authors used visual rating methods to measure MRI markers of silent cerebral lesions (SCLs). Location of these SCLs should be rated. Cognitive performance may be more related to the location of SCLs but not the types of SCLs.

REPLY
We agree with the reviewer. It would be interesting to assess the correlation between the location of SCLs and cognitive performance. However, the incidence and number of silent lacunes were lower than expected, making it not feasible to assess such correlation. We are interested to

investigate this in future study.

2. Authors did not mention the severity of SCLs of these participants. SCLs usually are mild in the normal population without vascular risk factors.

REPLY
We agree with the reviewer that the severity of SCLs of these participants is important. We did mention it and have discussed about it as limitations of our study.

Minor Concerns:
1. cognitive test " verbal influence" should be "verbal fluency”.

REPLY
We are grateful to the reviewer and have corrected the typo mistake.

2. A table for presenting the demographic data of this participants is suggested.

REPLY
We are grateful to the reviewer and have added a table for the demographic data (Table 3).

3. A table for the cognitive performance and MRI markers of SCLs is suggested.

REPLY
We are grateful to the reviewer and have added a table for the cognitive performance and a table for SCLs on MRI (Table 4 and Table 5).

4. Vascular risk factors should be included in the analysis.

REPLY
Vascular risk factors have been included in the analyses.