Supplemental Methods

Participants

Patients were eligible if they were over the age of 45, had a diagnosis of hypertension or diabetes, and at least one other vascular risk factor [1]. To be included patients also needed to be determined to be at risk for subcortical vascular cognitive impairment as specified in the literature [2, 3, 4]. Exclusion criteria included significant known large vessel cerebrovascular disease; a history of large vessel stroke or large vessel TIA; a current diagnosis of dementia; use of cholinesterase inhibitors or other dementia medications; or a pattern of symptoms consistent with Alzheimer's disease or other neurodegenerative disease etiologies that could lead to MCI or dementia (e.g., Parkinson's Disease, Multiple Sclerosis, Traumatic Brain Injury).

Procedures

Single photon emission computed tomography

An intravenous line was established in all subjects before SPECT imaging was performed. While lying supine with eyes closed in a dimly lit, quiet room, each subject received an intravenous injection of 30mCi of ^{99m}Tc-ECD (Neurolite, Dupont Pharma, USA). Ten minutes after this injection, brain SPECT was performed with a double-headed gamma camera (ADAC Vertex) with low energy, high resolution collimators. For each camera, projection data were obtained in a 128x128 matrix through 360^{o} rotation, where 30.0 cm zoom yields a pixel size of 0.23 cm in reconstructed images. Typical total counts of raw data were between 10 and 20 million. Full width at half maximum (FWHM) was set at 3.5 mm. Approximately forty minutes of scan acquisition commenced shortly thereafter.

SPECT Image Analysis using eZIS:

The age groups used for the normal control SPECT images for the eZIS analyses included 40-59 (19 men and 11 women), 60-69 (18 men and 22 women), and 70-86 (20 men and 20 women). The intensity value of each voxel in the patient's image was mapped to a Z score, using the mean and standard deviation calculated from the normal control images [5]. The Z score maps were displayed by overlay onto topographic sections and by projection, with an averaged Z score obtained from a depth of 14 mm to the surface through rendering of an anatomically standardized MRI template.

Statistical analysis:

Similar to Nobili et al. [6], correlation between the SPECT data and the neuropsychological scores were computed by statistical parametric mapping (SPM) [7], 2008 version (SPM8). In a preprocessing step, data sets were spatially normalized using a 12 point linear affined transformation into MNI Talairach space [8] and smoothed with an isotropic Gaussian kernel of FWHM 12mm. SPM8 was used to spatially normalize the individual SPECT images to the SPECT template provided with SPM. Since this template does not completely match the Talairach brain, a correction of the SPM{t} coordinates is needed. This was achieved using the subroutine implemented by Matthew Brett (http://www.mrc-cbu.cam.ac.uk/Imaging) which gives the correspondence between SPM and the Talairach atlas coordinates. Threshold masking was not used for the analyses. Z score images created from the eZIS program were subsequently analyzed using SPM8, using scores from cognitive tests and other measures as a covariate. The resulting SPM{t} were displayed using a threshold set at $p = 0.001$, which is an accepted procedure in a clinical setting [9].

References For Supplemental Methods

[1] Baker JG, Williams AJ, Ionita C, Lee-Kwen P, Ching M, Miletich RS. Cerebral small vessel disease: Cognition, mood, daily functioning, and imaging findings from a pilot sample. Dement Geriatr Cogn Disord Extra [in press].

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[3] Roman GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC. Subcortical ischaemic vascular dementia. Lancet Neurol 2002; 1: 426-36.

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[5] Waragai M, Yamada T, Matsuda H. Evaluation of brain perfusion SPECT using an easy Z score (eZIS) as an adjuct to early-diagnosis of neurodegenerative diseases. J Neurol Sci 2007; 260: 57-64.

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[7] Friston KJ, Frith CD, Liddle PF, Frackowiak RSJ. Comparing functional (PET) images: the assessment of significant change. J Cereb Blood Flow Metab 1991; 11: 690-9.

[8] Talairach J, Tournoux P. Co-planar stereotaxic atlas of the human brain: 3-Dimensional proportional system - an approach to cerebral imaging. New York: Thieme Medical Publishers; 1988.

[9] Desgranges B, Baron JC, de la Sayette V, Petit-Taboue' MC, Benali K, Landeau B, Lechevalier B, Eustache F. The neural substrates of memory systems impairment in Alzheimer's disease: A PET study of resting brain glucose utilization. Brain 1998; 121: 611-31.

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Supplemental Results

Supplemental Table 1 presents a summary of the SPECT clinical report findings and the neuropsychology test result findings for the 12 individual participants, similar to results presented in Baker et al. [1]. The extent of small vessel disease noted in the clinical report is listed for each participant. As can be seen in Supplemental Table 1, of the 11 participants with neuropsychology test domains that are below one standard deviation from the normative mean score, eight participants' scores include at least one domain that would be expected to be affected by cerebral small vessel disease (e.g., attention, speed of processing, executive function, and memory retrieval).

Supplemental Figure 1 displays sagittal, coronal, and transaxial views of regions of significant association between the RBANS List Learning subtest score, one of the subtests included in the RBANS Immediate Memory Index score, and SPECT regional cerebral blood flow Z scores from the eZIS imaging analysis for the 12 participants in the study. The upper half of Supplemental Figure 1 displays voxels above the threshold value of $p < 0.001$ that are associated with the RBANS List Learning subtest score throughout the sagittal, coronal, and transaxialslices of the entire "glass brain." The greyscale shading indicates the intensity of the voxels.

The lower half of Supplemental Figure 1 presents more anatomical detail, with clusters associated with RBANS List Learning scores overlaid on a single sagittal, coronal, and transaxial slice of a standardized MRI template. As can be seen in both the upper and lower halves of Supplemental Figure 1, and with reference to Table 1 in the main article, one of these clusters in

the right frontal lobe contains 1500 voxels, and is significant at $p < 0.001$, corrected for multiple comparisons.

The most intense voxel in this cluster is located in the inferior frontal gyrus, and corresponds to the Talairach coordinates (52, 28, 5), nearest to Brodmann Area 45. The arrowhead and cross hairs in Supplemental Figure 1 show the location of this voxel. This voxel has a Z score intensity of .835, and does not reach significance. The location of this cluster and the voxels it contains is also generally consistent with regions involved in the list learning memory task included in the RBANS Immediate Memory Index score.

Supplemental Case Study

Supplemental Figure 2 shows the raw SPECT image and Supplemental Figure 3 shows the results of the eZIS imaging analysis for a case example from Baker et al. [1] involving an 84 year old White female with 14 years of education. White matter information is masked out from the eZIS Z score image using a gray matter mask. The MRI clinical imaging report for this case described no significant intracranial abnormality, and no abnormality of perfusion. Nonetheless, the clinical report based on the raw SPECT image noted a mild to moderate multifocal pattern of hypoperfusion consistent with small vessel disease, including hypoperfusion in the anterior temporal cortices. No pattern of hypoperfusion was seen that would be suggestive of large vessel disease, neurodegenerative disease, or psychiatric disorder.

Supplemental Figure 3 shows regions of both statistically significant hypoperfusion (blue areas) and statistically significant hyperperfusion (red areas). Areas of decreased perfusion in the anterior temporal lobes can be seen in the first two rows of the right panel and in the lateral and inferior views of the right panel of Supplemental Figure 3, consistent with the clinical

SPECT report. Neuropsychology test results indicated some mild decreased performance on measures of executive function and the Attention Index score of the RBANS. The statistically significant increased perfusion that can be seen in the frontal region on the eZIS results in Supplemental Figure 3 was interpreted as a compensatory mechanism for difficulty with executive functioning identified on neuropsychology testing. The eZIS results shown in Supplemental Figure 3 illustrate the potential usefulness of this functional imaging analysis approach in identifying changes in SPECT perfusion associated with very mild changes in neuropsychology test performance.

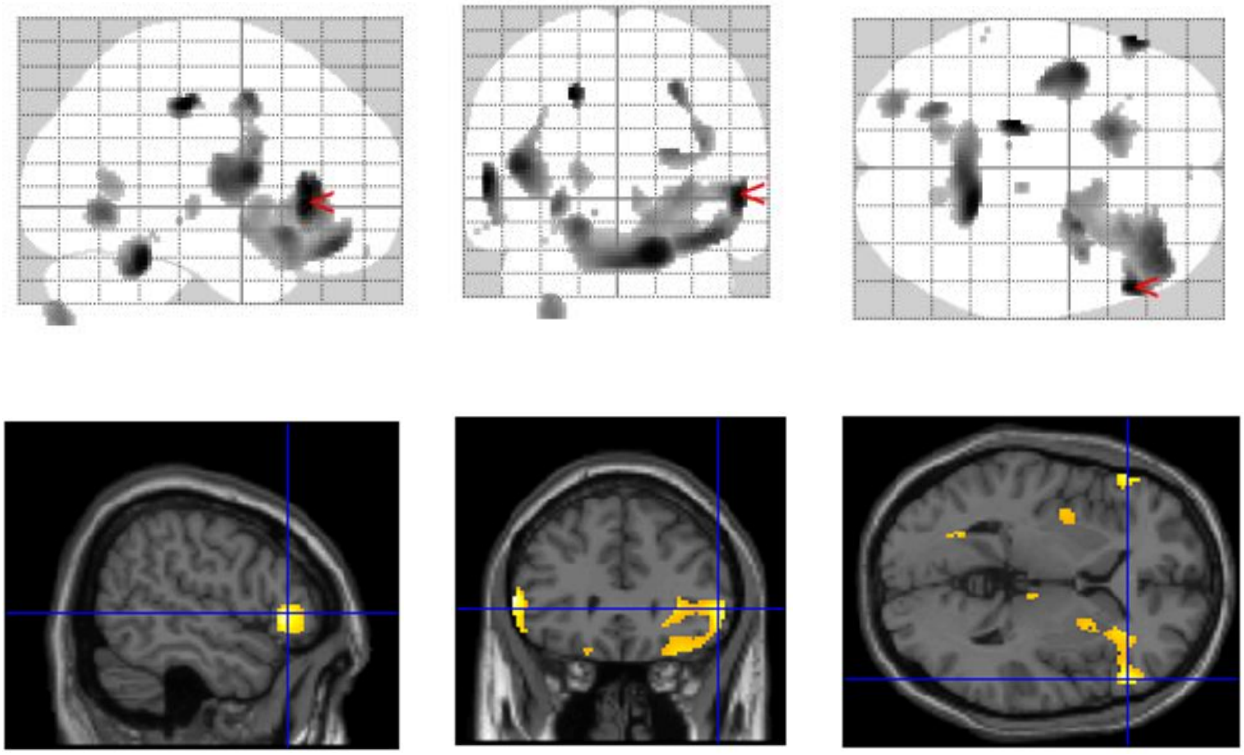
Supplemental Table 1. *Clinical report findings for SPECT imaging and neuropsychology test scores below one standard deviation*

	SPECT	Neuropsychological Testing
Case	Small Vessel Disease Hypoperfusion	Domains below 1 SD from the normative mean
1	Mild – Moderate	Attn, Del Mem[a]
2	Moderate	None [a]
3	Mild – Moderate	Attn, Speed [a]
4	Mild – Moderate	Del Mem, GDS, MCS
5	Mild – Moderate	Attn, Im & Del Mem, Lang, Ex Fx, GDS, MCS, PCS
7	Mild – Moderate	GDS, MCS, PCS
8	Mild – Moderate	Wk Mem, Ex Fx
9	Moderate	Im Mem, PCS
10	Mild – Moderate	GDS, MCS, PCS
11	Mild – Moderate	VS/Const
13	Incipient	Wk Mem, Ex Fx, MCS, PCS
14	Mild	Attn, Wk Mem, Im Mem, Lang, VS/Const, Ex Fx

[a]Tests of executive functioning were not administered

Y= Yes, N = No, Attn = Attention, Wk Mem = Working Memory, Im Mem = Immediate Memory, Del Mem = Delayed Memory, Speed = Processing Speed, Lang = Language, VS/Const = Visual Spatial/Constructional, Ex Fx = Executive Functioning, GDS = Geriatric Depression Scale, PCS = SF-36 Physical Composite Summary Score, MCS = SF-36 Mental/Emotional Composite Summary Score

Supplemental Figure 1.

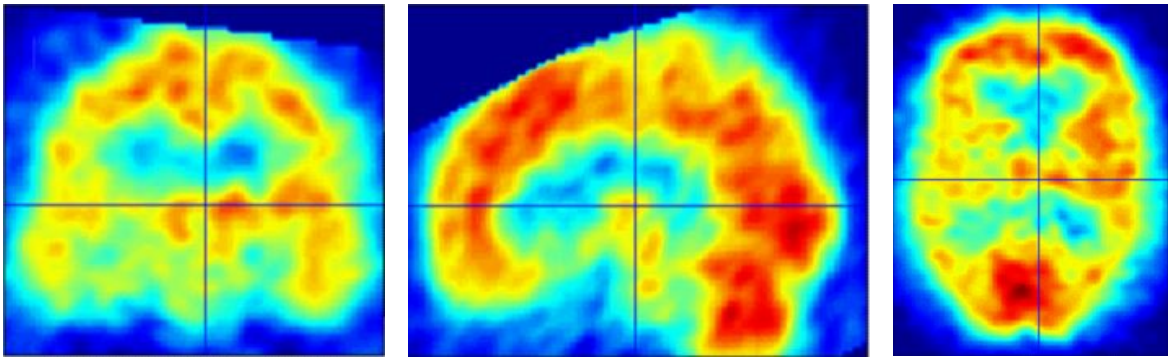


(a) (b) (c)

Z score map of SPM correlation (height threshold p = 0.001) between brain perfusion and score on the RBANS List Learning subtest. A significant area (shown both as glass brain and as regional cerebral blood flow) is found in the right frontal lobe. See Table 1 for cluster and voxel details (Talairach coordinates (52, 28, 5). (a) sagittal view; (b) coronal view; and (c) transaxial view.

Supplemental Figure 2.

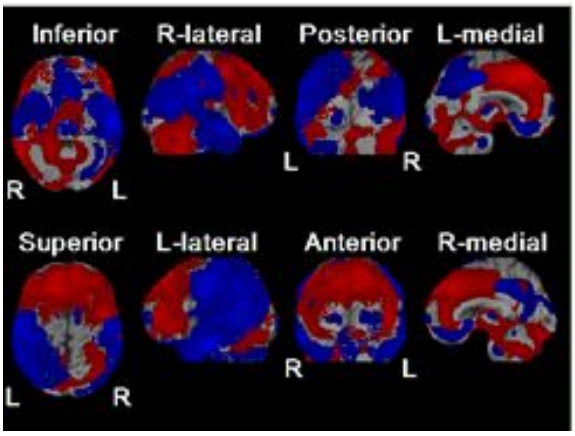
SPECT image of case example (84 year old White female with 14 years of education) with cerebral SVD: (a) coronal view; (b) sagittal view; and (c) transaxial view.

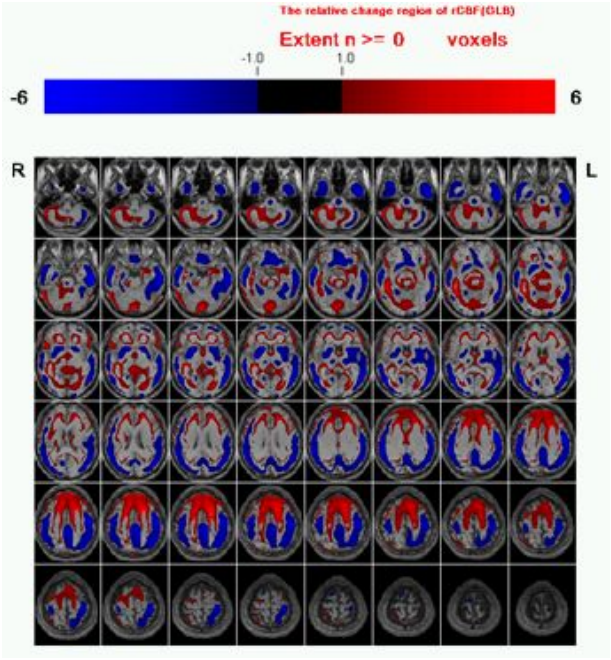


(a) (b) (c)

Supplemental Figure 3.

eZIS image of case example (84 year old White female with 14 years of education) with cerebral SVD (Left panel: surface view, Right panel: transaxial view).





References For Supplemental Results

[1] Baker JG, Williams AJ, Ionita C, Lee-Kwen P, Ching M, Miletich RS. Cerebral small vessel disease: Cognition, mood, daily functioning, and imaging findings from a pilot sample. Dement Geriatr Cogn Disord Extra 2012;2:169-179 (DOI: 10.1159/000333482) http://www.karger.com/Article/Pdf/333482.