Supplemental material

eMethod 1. Visual assessment and interrater agreement analysis for the identification of vessel-clusters on susceptibility-weighted imaging (SWI).

A stroke neurologist (SR) identified 114 regions of interest (ROI) indicating focal low-signal alterations in the white matter on SWI. These ROIs were characterized by lower signal intensity compared to the surrounding parenchyma showing either vessel-like structures or other nonspecific changes in SWI intensity of the white matter (i.e. for CSF content). Lesions characterized by pronounced blooming effect due to hemorrhagic content such as microbleeds or intracranial hemorrhages were excluded. The ROIs on SWI were manually outlined using ITK-SNAP software[1] including each slice on which each low signal area was visible, given a unique identity number, and evaluated on a radiological quality PACS image viewer (Carestream®). Then, two independent observers (SR and EC) evaluated the 114 ROIs to identify those compatible with the vessel-cluster definition, and counted the number of the vessel-like structures identified for each vessel-cluster. Interrater agreement between the two readers for the presence of vessel-clusters and number of vessel-like structure for each ROI was assessed with kappa statistics and weighted quadratic kappa-statistic, respectively,[2,3] and graded according to Altman criteria.[4] Interrater agreement for the presence of the clusters was substantial (kappa statistic = 0.66, 95% CI 0.49-0.84, $p<0.001$). Positive, negative, and global agreement (95% CI) were 93.5% (88.9%-96.2%), 72.7% (58.2%-83.7%), and 89.5% (82.5%-93.9%), respectively. The prevalence and bias adjusted kappa (PABAK) was 0.79. The interrater agreement for the number of single vessel-like structures in each cluster was also substantial (quadratic kappa statistic = 0.64, 95% CI 0.52-0.75, $p<0.001$).

eTable 1. Logistic multivariable regression for the presence of vessel-clusters in per-patient analysis

	Unadjusted OR (95% CI)	P	Adjusted OR (95% CI)	P
Age, y	0.98 (0.94-1.01)	0.221	0.94 (0.88-1.01)	0.090
Male sex	2.16 (0.86-5.44)	0.101	2.94 (0.84-10.34)	0.090
Alcohol use	2.49 (0.87-7.06)	0.088	1.52 (0.40-5.81)	0.543
CADASIL (vs. sporadic SVD)	4.20 (1.58-11.1)	0.004	2.34 (0.47-11.69)	0.300
Log 10 normalized WMH volume	2.31 (1.50-3.56)	<0.001	1.92 (1.04-3.56)	0.038
Number of lacunes	1.38 (1.16-1.64)	<0.001	1.30 (1.05-1.62)	0.018
Number of microbleeds	1.06 (0.99-1.13)	0.093	0.98 (0.90-1.08)	0.683
PVS (BG+CS) score	1.48 (1.13-1.95)	0.004	0.89 (0.56-1.41)	0.614
CVR in normal appearing white matter, %/mmHg*	0.87 (0.73-1.02)	0.094	0.77 (0.60-0.99)	0.040
CVR in WMH, %/mmHg*	0.91 (0.84-0.99)	0.026	0.90 (0.80-1.01)	0.069

Adjusted for age, sex, log 10 normalized WMH volume and number of lacunes, number of microbleeds, PVS (BG+CS) score, alcohol use, and SVD type. Analysis performed in the whole cohort (N=76), except for CVR variables that were available in 69 patients (*). WMH: white matter hyperintensities; CADASIL: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; BG: basal ganglia; CSO: centrum semiovale; PVS: perivascular spaces.

eTable 2. Ordinal multivariable regression for the number of vessel-clusters in per-patient analysis

	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Age, y	0.97 (0.93-1.00)	0.082	0.94 (0.88-0.99)	0.037
Male sex	2.48 (1.03-5.95)	0.043	3.63 (1.29-10.24)	0.015
Alcohol use	2.44 (0.82-6.18)	0.118	1.07 (0.34-3.33)	0.906
CADASIL (vs. sporadic SVD)	4.90 (1.97-12.0)	0.004	2.41 (0.62-9.31)	0.202
Log 10 normalized WMH volume	2.33 (1.59-3.40)	<0.001	1.80 (1.09-2.96)	0.021
Number of lacunes	1.26 (1.13-1.40)	<0.001	1.22 (1.03-5.31)	0.002
Number of microbleeds	1.06 (1.01-1.12)	0.031	0.97 (0.91-1.04)	0.369
PVS (BG+CS) score	1.50 (1.18-1.90)	0.001	1.06 (0.73-1.54)	0.744
CVR in normal appearing white matter, %/mmHg*	0.92 (0.81-1.03)	0.094	0.88 (0.77-1.01)	0.069
CVR in WMH, %/mmHg*	0.92 (0.87-0.99)	0.020	0.93 (0.86-1.01)	0.088

Adjusted for age, sex, log 10 normalized WMH volume and number of lacunes, number of microbleeds, PVS (BG+CS) score, alcohol use, and SVD type. Analysis performed in the whole cohort (N=76), except for CVR variables that were available in 69 patients (*). WMH: white matter hyperintensities; CADASIL: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; BG: basal ganglia; CSO: centrum semiovale; PVS: perivascular spaces.

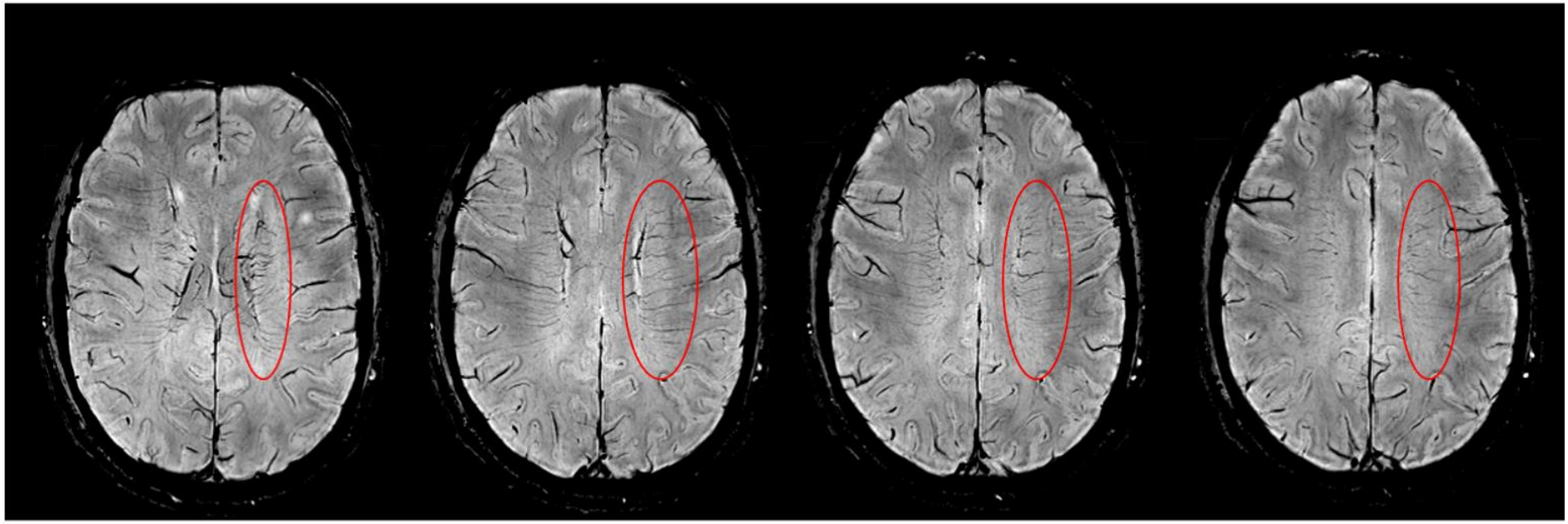
eTable 3. Structural features of the all vessel-clusters in per cluster analysis, and according to the presence of full cavitation (vs. no or partial cavitation), type of SVD (sporadic SVDvs. CADASIL), and number of lacunes (<8 vs. ≥8 lacunes)

	All vessel-clusters (N=94)	Vessel-clusters with full cavitation (N=37)	Vessel-clusters with partial or no cavitation (N=57)	P value	Vessel-clusters in sporadic SVD (N=33)	Vessel-clusters in CADASIL (N=61)	P value	Vessel-clusters in patients with <8 lacunes (N=42)	Vessel-clusters in patients with ≥ 8 lacunes (N=41)	P value
Number of vessel-like structures, median (IQR)	2 (1-3)	2 (2-3)	2 (1-3)	0.023	2 (1-3)	2 (1-3)	0.225	2 (1-3)	2 (1-3)	0.440
Side (left hemisphere), n (%)	48 (51)	20 (54)	28 (49)	0.640	15 (45)	31 (51)	0.619	20 (48)	26 (50)	0.818
Location in white matter, n (%)				0.219	13 (39)	28 (46)	0.188			0.121
Anterior	22 (23.4)	12 (32)	10 (18)		10 (30)	12 (20)		6 (14)	16 (31)	
Middle	55 (58.5)	20 (54)	35 (61)		60 (61)	35 (57)		29 (69)	26 (50)	
Posterior	17 (18.1)	5 (13)	12 (21)		3 (9)	14 (23)		7 (17)	10 (19)	
Vessel-cluster region shape				0.153			0.827			0.730
Round	45 (47.9)	18 (49)	27 (47)		16 (48)	29 (48)		19 (45)	26 (50)	
Ovoid	32 (34.0)	15 (41)	17 (30)		11 (33)	21 (34)		14 (33)	18 (35)	
Irregular	6 (6.4)	3 (8)	3 (5)		3 (9)	3 (5)		4 (10)	2 (4)	
Linear	11 (11.7)	1 (3)	10 (18)		3 (9)	8 (13)		5 (12)	6 (12)	
Linear rim, n (%)	41 (43.6)	26 (70)	15 (26)	<0.001				10 (24)	31 (60)	0.001
Vessel-cluster region volume, mL, median (IQR)	0.150 (0.082-0.257)	0.237 (0.188-0.377)	0.105 (0.068-0.165)	<0.001	0.168 (0.082-0.259)	0.150 (0.084-0.233)	0.779	0.128 (0.044-0.209)	0.168 (0.099-0.275)	0.125

eTable 4. Per-cluster cerebrovascular reactivity (CVR) magnitude analysis in vessel-cluster volumes and surrounding tissue using contralateral volumes as reference.

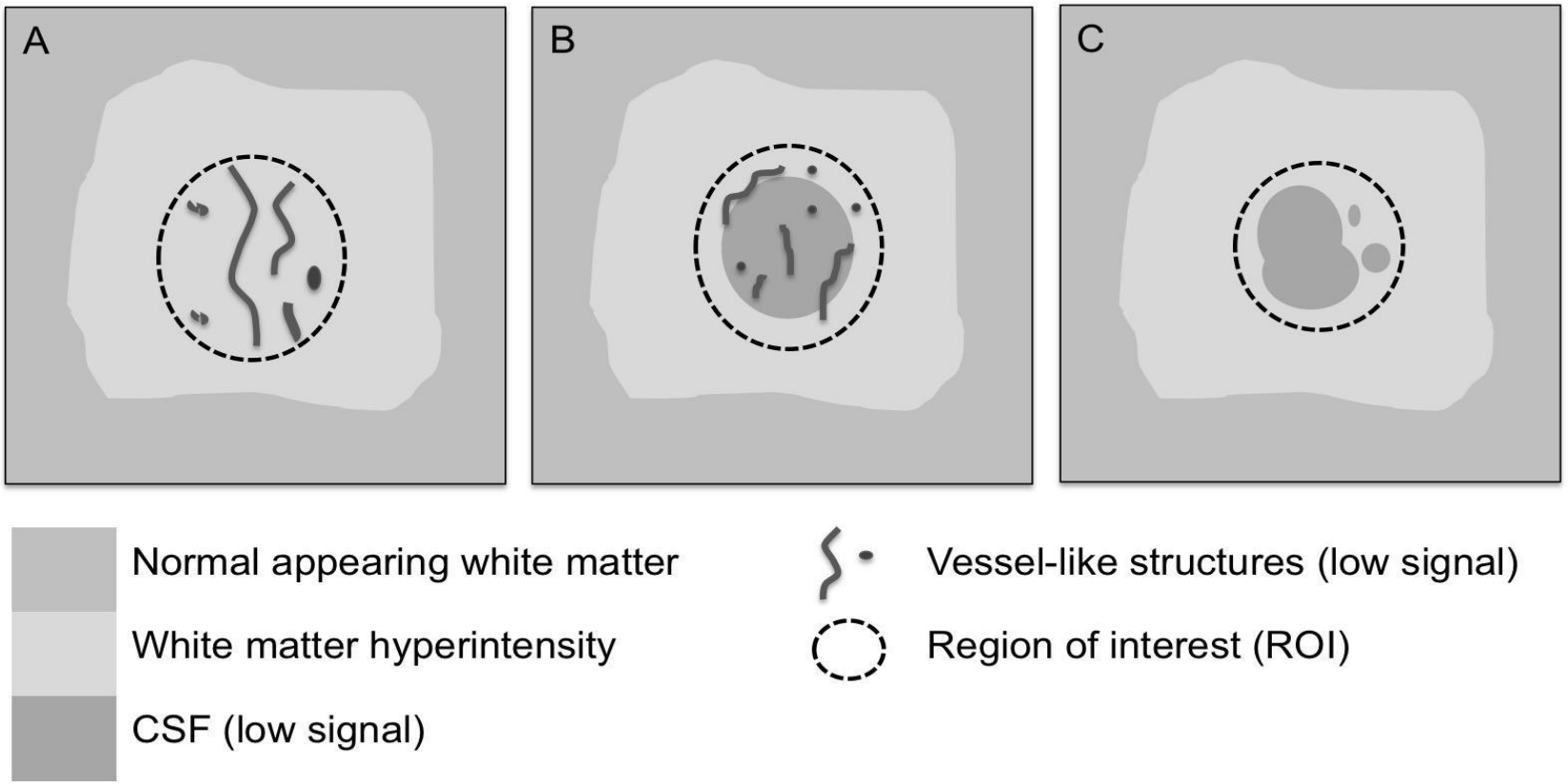
	Vessel cluster CVR (%/mmHg)	Contralateral volume CVR (%/mmHg)	Mean difference (SD) (%/mmHg)	2 sample t test, t	P value
All vessel-clusters					
Vessel cluster	0.012 (0.118)	0.033 (0.086)	-0.021 (0.119)	-1.528	0.131
2 voxel volume expansion	0.016 (0.074)	0.022 (0.057)	-0.006 (0.043)	-1.341	0.183
4 voxel volume expansion	0.017 (0.062)	0.026 (0.054)	-0.008 (0.040)	-2.047	0.044
6 voxel volume expansion	0.026 (0.054)	0.026 (0.053)	0.000 (0.028)	0.047	0.963
0-2 voxel shell	0.015 (0.073)	0.021 (0.057)	-0.006 (0.043)	-1.330	0.187
2-4 voxel shell	0.017 (0.062)	0.025 (0.054)	-0.008 (0.040)	-2.039	0.044
4-6 voxel shell	0.026 (0.054)	0.026 (0.052)	0.000 (0.028)	0.066	0.948
Vessel-clusters with full cavitation					
Vessel cluster	-0.023 (0.089)	0.023 (0.094)	-0.046 (0.088)	-3.021	0.005
2 voxel volume expansion	0.008 (0.070)	0.019 (0.063)	-0.011 (0.031)	-2.140	0.039
4 voxel volume expansion	0.011 (0.057)	0.021 (0.057)	-0.010 (0.027)	-2.295	0.028
6 voxel volume expansion	0.018 (0.052)	0.021 (0.051)	-0.003 (0.030)	-0.697	0.490
0-2 voxel shell	0.006 (0.070)	0.017 (0.063)	-0.011 (0.033)	-2.039	0.049
2-4 voxel shell	0.010 (0.056)	0.020 (0.057)	-0.010 (0.027)	-.2.234	0.026
4-6 voxel shell	0.017 (0.052)	0.021 (0.051)	-0.004 (0.030)	-0.723	0.474
Vessel-clusters with partial or no cavitation					
Vessel cluster	.004 (0.132)	0.041 (0.079)	-0.001 (0.136)	-0.031	0.976
2 voxel volume expansion	0.021 (0.076)	0.023 (0.053)	-0.003 (0.049)	-0.416	0.679
4 voxel volume expansion	0.021 (0.065)	0.028 (0.052)	-0.007 (0.047)	-1.200	0.235
6 voxel volume expansion	0.032 (0.055)	0.029 (0.054)	0.002 (0.026)	0.682	0.498
0-2 voxel shell	0.021 (0.076)	0.023 (0.053)	-0.003 (0.049)	-0.405	0.687
2-4 voxel shell	0.021 (0.065)	0.028 (0.052)	-0.007 (0.047)	-1.176	0.244
4-6 voxel shell	0.032 (0.055)	0.029 (0.054)	0.003 (0.026)	0.730	0.468

eFigure 1. Normal appearance of deep medullary veins on SWI in a patient with sporadic small vessel disease (SVD).



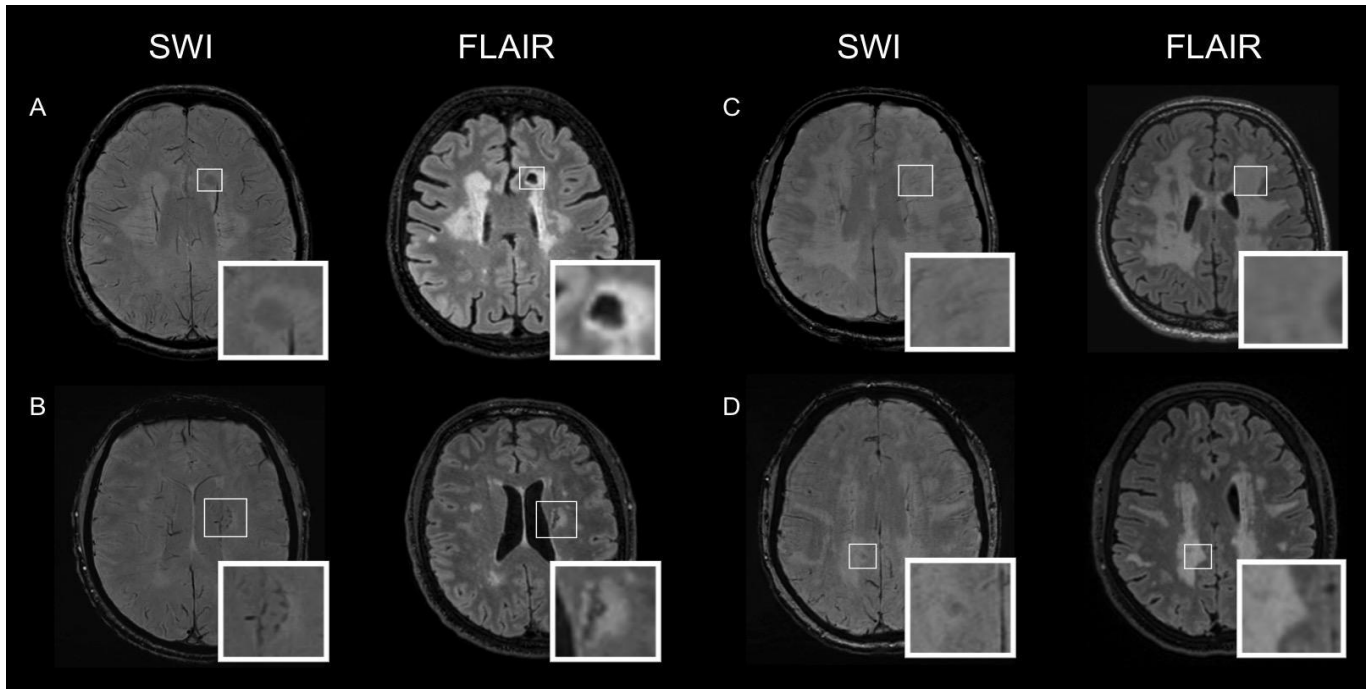
In the figure 4 consecutive (from caudal to rostral) axial images from SWI acquisition of a patient with sporadic SVD. The red circles show in the left centrum semiovale the normal appearance of the white matter venous drainage from the deep medullary veins (perpendicular to the lateral ventricles) to the corresponding subependymal veins (parallel to the lateral edge of the lateral ventricle) and finally converging into the deep venous system.

eFigure 2. Schematic representation of the finding on SWI at visual assessment for the identification of the vessel-clusters.



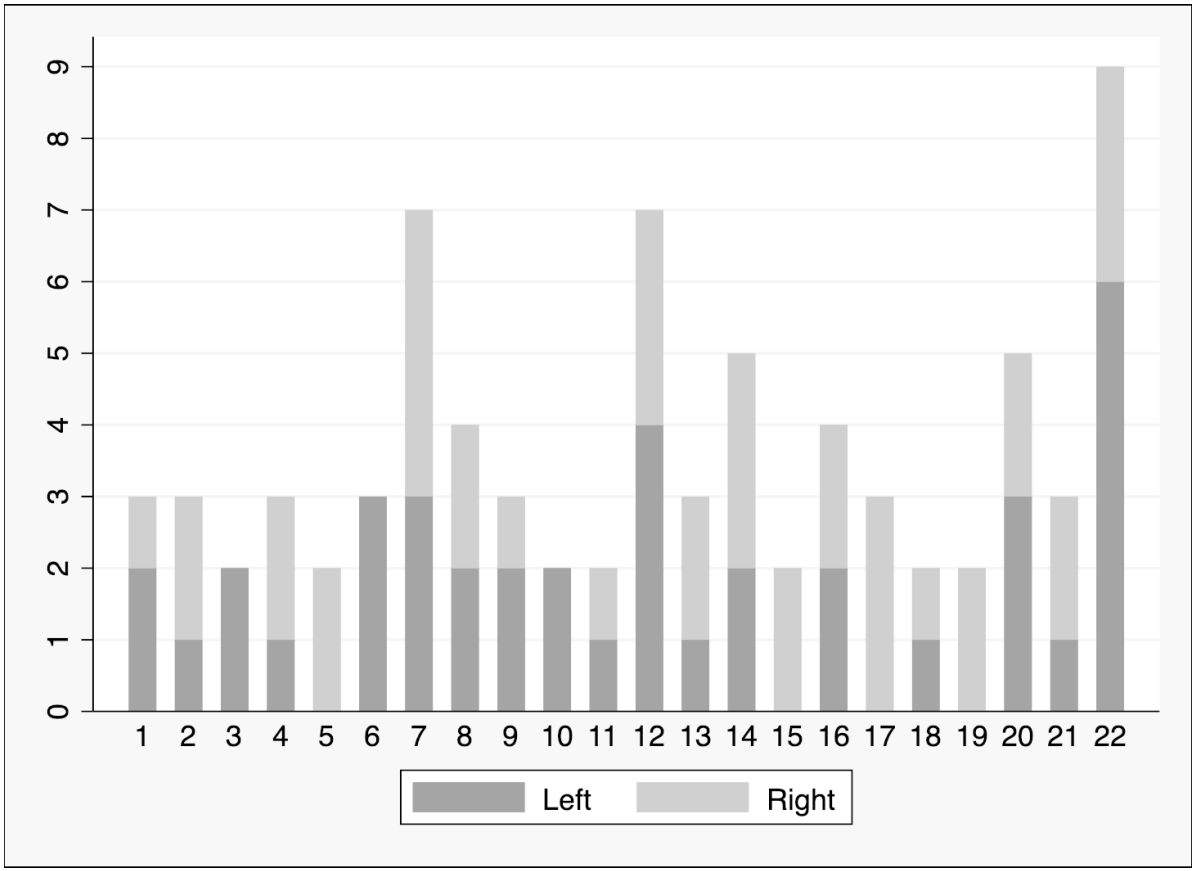
A: tubular and/or punctate vessel-like low signal structures on SWI may be visible in a focal ROI within white matter hyperintensity (WMH) areas. B: vessel-clusters may appear within or on the edge of regions with lower signal compared to the surrounding tissue and corresponding in most cases to cavities containing CSF (i.e. lacune). C: a ROI may show CSF-like appearance but no vessel-clusters are visible.

eFigure 3. Examples of disagreements in visual assessment on SWI for vessel-clusters. Equivocal appearances of vessels (e.g., “loop-like” shapes or branching structures, proximity to periventricular veins, and images with very small diameters) gave rise to the majority of discordances in visual assessment results between the two readers. Small cavities with CSF may also be difficult to differentiate from vessel-like structures. FLAIR sequences were not assessed for the vessel-cluster agreement, but are also shown on the panel to show the structural features corresponding to the regions of interest evaluated.



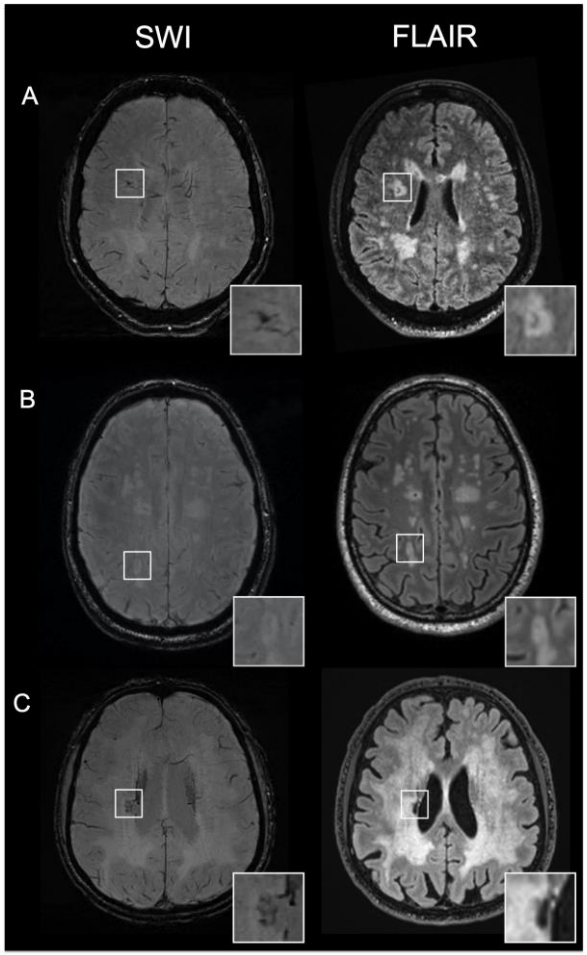
A: a left periventricular round low-signal CSF-like image is visible on SWI, corresponding to a complete lacune on FLAIR. A big vein (longitudinal caudate vein of Schlesinger) is reaching the edge of the lacune, but no small vessels were observed in the edges of the lacune. B: small dilated vessels are shown in the lateral edge of an incomplete lacunes within focal WMH, while in the medial edge the dilated vessel corresponds to the transverse caudate vein. C: some vessels appear prominent in the WMH but do not clearly differentiate from deep medullary veins. Although these findings could be related to initial phases of vessel-clusters formation, they were not considered as vessel-clusters because of very unspecific appearance at visual assessment. D: a right frontal parasagittal low-signal image is visible on SWI corresponding to WMH, but no small vessels within the areas were clearly identified.

eFigure 4. Hemisphere affected by vessel-clusters in the 22/76 patients with more than one vessel-cluster (29%)



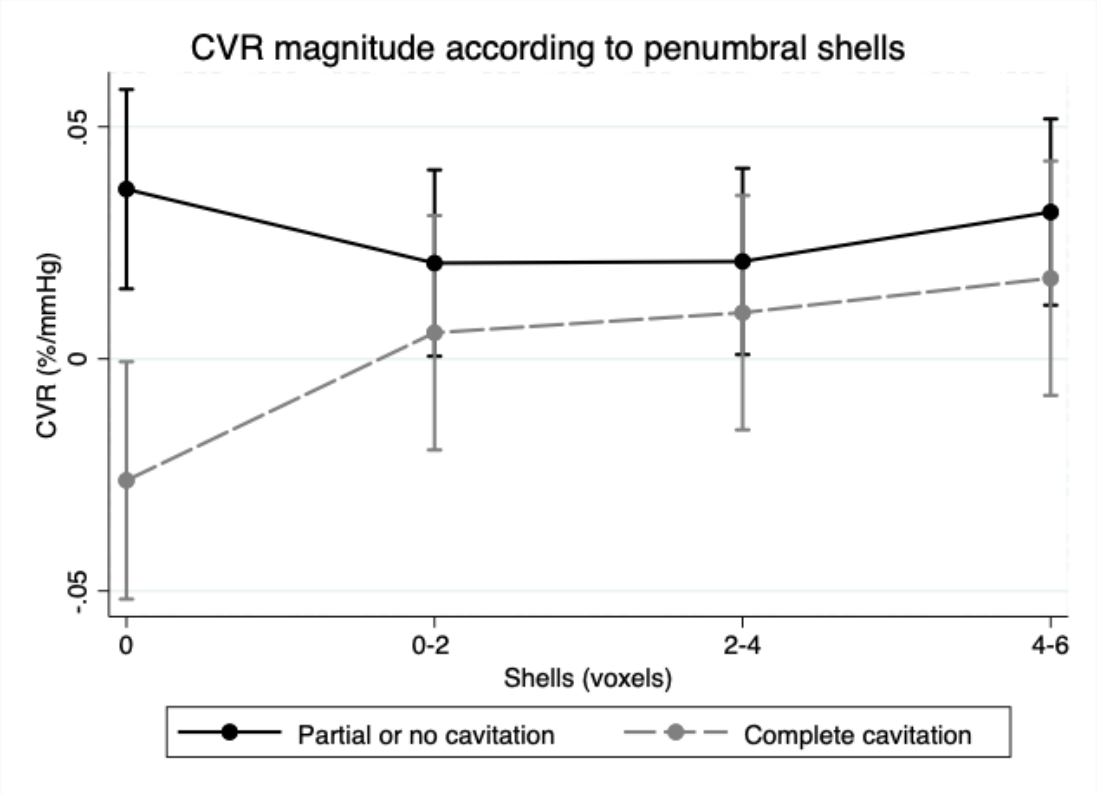
The bar chart shows as the vessel-clusters had a predominantly symmetrical distribution in those patients who had more than one vessel-cluster. Y axis: number of vessel-clusters per patients, X axis: each bar represents a patient with more than one vessel-cluster. Dark and light gray represent vessel-clusters in the left and right hemispheres, respectively.

eFigure 5. Examples of vessel-clusters presenting different morphologic features in 3 patients with CADASIL.



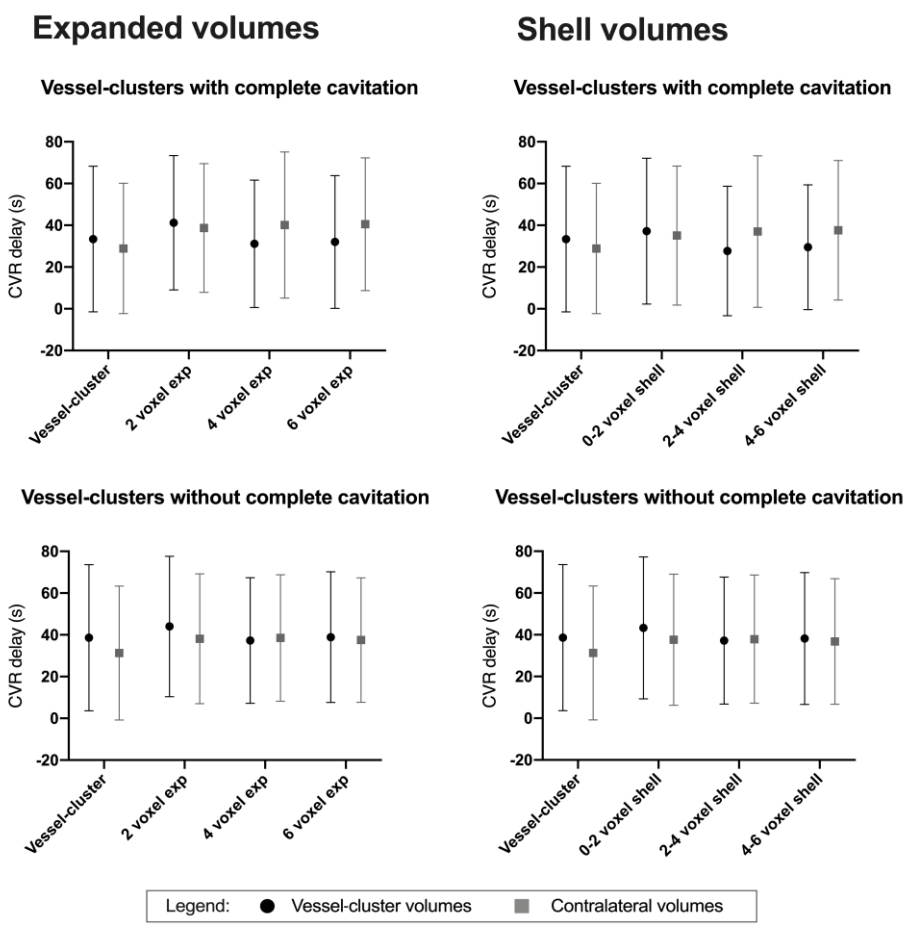
A: various vessels seem to be draining from an incomplete lacune to the lateral ventricle. B: a single dilated vessel is visible in the middle of a focal WMH area without cavitation. C: some dilated vessels appear in the WMH boarding the lateral edge of a periventricular lacune.

eFigure 6. Gradient effect of CVR towards concentric penumbral shells.



Points represent mean CVR and bars 95% CI. Linear significant trend in increasing CVR is shown in the subgroup of cluster with complete cavitation (gray marks, dashed line), while no trend relationship was present in vessel-clusters with partial or no cavitation (black marks, continuous line).

eFigure 7. Per-cluster cerebrovascular reactivity (CVR) delay analysis compared to contralateral volumes.



Forest plots: Black points (cluster-vessels volumes) and grey squares (contralateral volumes) represent mean delay (s, seconds) and bars represent standard deviation. None of the comparisons was significant ($p<0.05$). On the left the charts represent CVR-delay within concentrical expansions the vessel-cluster volume (including it), on the right side of the panel the charts represent CVR-delay within the original vessel-cluster volume and concentrical penumbral shells.

eReferences

1. Yushkevich PA, Pashchinskiy A, Oguz I, et al. User-Guided Segmentation of Multi-modality Medical Imaging Datasets with ITK-SNAP. Neuroinformatics. 2019;17:83–102.
2. Cohen J. A Coefficient of Agreement for Nominal Scales. Educ Psychol Meas. 1960 [cited 2021 Apr 3];20:37–46.
3. Cohen J. Multiple regression as a general data-analytic system. Psychol Bull. 1968 [cited 2021 Apr 3];70:426–443.
4. Chapmans AD. No Title. In: Practical statistics for medical research. Chapmans H. London; 1991. p. 403–409.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	Secondary cross-sectional analysis of a prospective multicentre observational study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1	We assessed their frequency, associations with SVD lesions and vascular reactivity in patients with. /// we identified 94 vessel-clusters in 36/76 patients /// CVR magnitude was lower than in corresponding contralateral volumes
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4	Upon inspection of high-quality MRI scans from patients with severe SVD, we recently observed small clusters of linear-like structures in deep WMH
Objectives	3	State specific objectives, including any prespecified hypotheses	4	We hypothesized that these may represent grouped small dilated vessels associated with white matter injury and cavitation (i.e. lacunes). In the current study, we describe the prevalence and characteristics of these possible clusters of small dilated vessels on SWI, their associations with patient demographics, SVD lesions and measures of vascular reactivity, in patients with sporadic SVD or with monogenetic SVD
Methods				
Study design	4	Present key elements of study design early in the paper	4	This is a secondary cross-sectional analysis from a prospective multicentre observational study of patients with symptomatic sporadic or genetic SVD
Setting	5	Describe the setting, locations, and relevant dates, including	5	The patients with sporadic SVD were recruited from centres in Edinburgh (UK) and Maastricht (the

		periods of recruitment, exposure, follow-up, and data collection		Netherlands), and patients with CADASIL from Munich (Germany).
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5	The INVESTIGATE-SVD study cohort included patients with symptomatic sporadic (a lacunar ischaemic stroke in the last 5 years or vascular cognitive impairment with SVD) or genetic SVD (diagnosis of CADASIL) [9] Patients with other causes of stroke such as ≥50% luminal stenosis, major-risk cardioembolic source of embolism (i.e. atrial fibrillation) and other specific causes of stroke identified (i.e. haemorrhage, arteritis, etc.) were not enrolled in the study.[9]
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6	Radiological markers of SVD, i.e. lacunes, PVS, microbleeds, and WMH on structural MRI sequences were identified according to STRIVE criteria,[2] and graded using validated qualitative scales for WMH (Fazekas scale)[10] and PVS load scale in basal ganglia and centrum semiovale.[11] All image analysis was centralized and conducted by an analyst not involved in the clinical assessments and masked to patient characteristics and CVR results and performed prior to the assessment of this study. We defined as "vessel-clusters"
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment	*5*	The full image acquisition protocol has been previously published.[9]

		methods if there is more than one group		
Bias	9	Describe any efforts to address potential sources of bias	6	For the analysis of CVR magnitude and delay within normal appearing white matter and WMH, we eroded the outer margin of the original white matter mask by 2.5mm (1 voxel) to reduce the influence of partial volume effects. Additionally, we masked the images with a dilated ventricle mask to exclude contamination from ventricular CSF and normal vessels running along the ventricle walls.
Study size	10	Explain how the study size was arrived at	5	The full image acquisition protocol has been previously published.[9]
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7	to assess CVR magnitude and delay in the vessel-clusters and in the surrounding tissue, from the original vessel-cluster segmentations we generated 3 additional concentric circumferential 3D expansions (shells) around the vessel-clusters, each 2-voxels thick in T2-w space (which approximates 1 voxel in CVR data) limited to the white matter, as represented in Figure 2. Then we automatically generated contralateral-mirrored segments within the white matter, checked these for accurate mirror-image location and edited manually if required (EC and SR). FLS software[16] was used for the mask processing.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7	*Statistical analysis*
		(*b*) Describe any methods used to examine subgroups and interactions	8	In per-cluster analysis

		(*c*) Explain how missing data were addressed	7	No missing values were detected among variables, except for systolic and diastolic blood pressure at the time of MRI that were not available in in 6/76 patients (8%), and CVR data were not usable in 7/76 patients (9%).
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy		
		(*e*) Describe any sensitivity analyses		
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9	Seventy-seven patients were recruited in the INVESTIGATE-SVD study from November 2017 to September 2019. Patients with sporadic SVD were recruited in Edinburgh (n=25) and Maastricht (n=20), and patients with CADASIL in Munich (n=32). All patients completed the main structural sequences.
		(b) Give reasons for non-participation at each stage	8	However, SWI sequences from one CADASIL patient were seriously affected by movement artifacts and that patient was excluded from the analysis
		© Consider use of a flow diagram		
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8	Detailed information of clinical and radiological features of the study cohort is summarized in Table 1.

		(b) Indicate number of participants with missing data for each variable of interest	7	No missing values were detected among variables, except for systolic and diastolic blood pressure at the time of MRI that were not available in in 6/76 patients (8%), and CVR data were not usable in 7/76 patients (9%).
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)		
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time		
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures	*9*	Thirty-six of 76 patients (47%) showed at least one vessel-cluster
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9	In the multivariable analysis, CADASIL subtype and alcohol use were no longer associated with the presence of vessel-clusters, and among structural imaging variables only the number of lacunes (OR=1.30; 95% CI, 1.04-1.62; p= 0.018) and normalized log10 WMH volume value (OR=1.92; 95% CI, 1.04-3.56; p=0.038) remained significant (Figure 3).
		(*b*) Report category boundaries when continuous variables were categorized		
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period		
Other analyses	17	Report other analyses done—eg analyses of subgroups and	10	A total of 94 vessel-clusters were identified amongst 36 patients

		interactions, and sensitivity analyses		
Discussion				
Key results	18	Summarise key results with reference to study objectives	11	The vessel-clusters were more likely to be seen in patients with large WMH volume and were highly associated with both the overall number of lacunes and co-located with what appeared to be cavities at different stages of formation.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14	This study also has some limitations
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15	The properties of deoxygenated blood on susceptibility-weighted sequences and the association with lower CVR in the white matter and in the surrounding tissue in clusters with cavities suggest that the vessel-clusters represent maximal a) dilatation of small deep vessels and b) oxygen extraction in white matter that is approaching terminal injury and cavitation.
Generalisability	21	Discuss the generalisability (external validity) of the study results	15	Finally, the presence of vessel-clusters has not been assessed in a control group without SVD. However, the likelihood of finding vessel-clusters in brains without small vessel lesions is expected to be extremely low
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for	16	SR receives funding from Instituto de Salud Carlos III, with a grant for health research and a mobility grant (CM18/00116; RH041992). INVESTIGATE@SVDs is funded by the European Union Horizon 2020, PHC-03-15, project No 666881, 'SVDs@Target'.

	the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.