Title: A systematic review of dynamic cerebral and peripheral endothelial function in lacunar stroke versus controls.

Authors: Susan F Stevenson, BSc (Hons)

Fergus N Doubal, MB ChB, MRCP

Kirsten Shuler, BSc

Joanna M Wardlaw, FRCP, FRCR, FMedSci

Affiliation: SINAPSE Collaboration, SFC Brain Imaging Research Centre, Division of Clinical Neurosciences, University of Edinburgh, Edinburgh, UK.

Correspondence: Prof J Wardlaw, SINAPSE Collaboration, SFC Brain Imaging Research Centre, Division of Clinical Neurosciences, Western General Hospital, Crewe Rd, Edinburgh, EH4 2XU, UK.

Tel: +44 131 537 2943

Fax: +44 131 332 5150

Email: Joanna.wardlaw@ed.ac.uk

Acknowledgements and Source(s) of Funding

The work was undertaken as part of a Special Study Module, College of Medicine and Veterinary Medicine, University of Edinburgh, UK. FD was funded by a Wellcome Trust Research Training Fellowship (075611). JMW is funded by the Scottish Funding Council and Chief Scientist Office as part of the SINAPSE (Scottish Imaging Network - Platform for Scientific Excellence, www.sinapse.ac.uk) Collaboration.

Conflict(s) of Interest/Disclosure(s)

The authors report no conflicts of interest.

Title: A systematic review of dynamic cerebral and peripheral endothelial function in lacunar stroke versus controls.

Cover title: Endothelial dysfunction in lacunar stroke

Word count: abstract 223, body of text 2346, references 1226; table 945 (on line); total 4741.

Total number of figures: 2

Total number of tables: 1

Key words: atherosclerosis, endothelium, plethysmography, stroke, vascular diseases

Abstract

Background: The aetiology of cerebral small vessel disease is unknown. An association with endothelial dysfunction has been suggested. We systematically assessed all relevant studies of dynamic endothelial function in patients with lacunar stroke, as a marker of small vessel disease.

Methods: We searched for studies of cerebral or peripheral vascular reactivity in patients with lacunar or cortical (i.e. large artery atheromatous) ischaemic stroke or non-stroke controls. We calculated standardised mean difference (SMD) in vascular reactivity, +/- 95% confidence intervals (CI) between small vessel disease and control groups.

Results: Sixteen publications (974 patients) were included. In lacunar stroke: cerebrovascular reactivity (n=534) was reduced compared with age-matched normal (SMD -0.94, 95%CI -1.17, -0.70), but not age+risk factor-matched controls (SMD 0.08, 95%CI -0.36, 0.53) or cortical strokes (SMD -0.29, 95%CI -0.69, 0.11); forearm flow mediated dilatation (n=401) was reduced compared with age-matched normal controls (SMD -1.04, 95%CI -1.33, -0.75) and age+risk factor-matched controls (SMD -0.94, 95%CI -1.26, -0.61), but not cortical strokes (SMD -0.23, 95%CI -0.55, 0.08).

Conclusions: Endothelial dysfunction is present in patients with lacunar stroke but may simply reflect exposure to vascular risk factors and having a stroke, as a similar degree of dysfunction is found in cortical (large artery atheromatous) stroke. Current data do not confirm that endothelial dysfunction is specific to small vessel stroke. Future studies should include controls with non-lacunar stroke.

Introduction

Cerebral small vessel disease (SVD) is common and has clinical (lacunar stroke, cognitive impairment, gait and movement disorders) and radiological (symptomatic small subcortical infarct, silent lacunar infarct, lacunes, white matter lesions (WMLs), microbleeds) manifestations.[1-3] The low early mortality of lacunar stroke masks a long-term risk of recurrent stroke and death similar to atherothromboembolic stroke,[4] and substantial risk of cognitive decline,[5] creating a massive public health burden.[6]

Lacunar infarcts are small (<15mm) subcortical lesions in the territory of a single deep perforating arteriole most of which are associated with an intrinsic abnormality in the perforating arteriole wall of unknown aetiology. An association between lacunar ischaemic stroke and endothelial dysfunction has been suggested,[7;8] but many patients with stroke have hypertension or diabetes or take medications which affect endothelial function.[9;10] Atheromatous large artery disease is also associated with endothelial dysfunction.[11] A recent systematic review identified endothelial dysfunction in lacunar ischaemic stroke but did not control for risk factor exposure or other stroke subtypes.[12] Therefore it is unclear whether endothelial changes observed in patients with lacunar ischaemic stroke might be specific to SVD or simply reflect age, vascular risk factors, generalised (possibly co-incidental) atheroma or the effects of having a stroke. We performed a systematic review of all studies that assessed cerebral or peripheral vascular reactivity in patients with lacunar ischaemic stroke.

Methods

We followed the general guidance for systematic reviews of observational and diagnostic studies (http://www.equator-network.org), modified to suit the type of study identified in this review.[13;14]

We searched the published literature using MEDLINE and EMBASE from the 1st January 1995, to the 15th February 2008 using Ovid and a carefully devised search strategy (Appendix 1) developed with advice from the Cochrane Stroke Group (http://www.dcn.ed.ac.uk/csrg/). We updated the search using MEDLINE to 6th January 2010 (we did not re-search EMBASE as there was very little difference between it and MEDLINE in the initial search). We sought primary studies, in humans, in any language, which investigated patients with markers of cerebral SVD and dynamic measures of endothelial function e.g. response to hypercapnia or acetazolamide or flow mediated dilatation.[15] We checked references in review and primary papers and hand-searched the journal *Stroke.*

We included papers which assessed endothelial function in patients with: clinically-evident lacunar ischaemic stroke, with or without an acute subcortical infarct on brain imaging; lacunes (i.e. rounded cerebral spinal fluid attenuation lesion <1.5mm in the basal ganglia, hemispheric white matter or brain stem) identified on brain imaging without clearly-relevant symptoms; or leukoaraiosis (WMLs).

We excluded papers which only assessed endothelial function using plasma markers, animal studies, duplicate publications, SVD caused by a single gene disorder or had no control group.

Two reviewers independently extracted data using a standardised data extraction form. A third reviewer arbitrated on disagreements. We obtained translations of foreign language papers where possible. We extracted data on study population (sample size, age, sex, presence of co-morbidities, medications, previous strokes and selection criteria for both patient and control groups), study design, endothelial function assessment method,[15] vascular bed, blinding of investigators and primary vascular reactivity results. We identified the method of stroke diagnosis, whether by a stroke specialist, if confirmed using imaging, the type of imaging and the time interval after stroke. We assessed the study quality using the QUADAS[14] instrument (www.equator-network.org). We were careful to include each patient population only once while including all data available for that population.

The studies used different methods of assessing endothelial function, so we calculated the standardised mean difference (SMD) by the fixed effects method (Review Manager 4© software) between the lacunar and control groups from the mean and standard deviation of the vascular reactivity results (where given). We used the endothelial function data from the cerebral circulation contralateral to the side of the symptomatic infarct in cortical and lacunar stroke patients. As most studies did not adjust for potential confounders, we stratified the analyses into lacunar vs. age-matched controls; vs. age+risk factor-matched controls; vs. cortical stroke; and multiple vs. single lacunar infarcts on imaging. We calculated heterogeneity between studies.

Results

We identified 1257 titles. De-duplication (467) and exclusion of irrelevant papers (n=739) resulted in 27 includable papers. Hand searching identified three additional publications. Of the 30 for full text reading, three were excluded (unable to translate: Russian[16] and Slovakian[17;18]) and eleven failed to meet the inclusion criteria (no controls,[19;20] not assessing dynamic endothelial function or inappropriate patients[21-29]). Therefore 16 papers were eligible, including 974 individuals.

Characteristics of included studies (Table 1)

Some of the 16 papers contributed to more than one comparison: 14/16 papers[30-43] compared 318 patients with recent lacunar ischaemic stroke to 305 age-matched or age+risk factor matched controls; 5/15 papers[35;37;38;42;44] compared 124 patients with recent lacunar ischaemic stroke to 115 patients with recent cortical ischaemic stroke; 4/15 papers[30;37;40;45] compared patients with recent lacunar ischaemic stroke of whom 71 had only one single small subcortical infarct and 71 had a single symptomatic lacunar infarct plus multiple silent infarctions on imaging.

Most studies were small (mean 36 subjects, median 18 subjects per group), gave little detail about recruitment or methods of stroke diagnosis (in particular of how lacunar stroke was determined), did not state duration of hypertension or other risk factors, adjust for risk factors[37] or mention prescribed medications (only two stated that medications were discontinued prior to study[35;40]). Only two studies explicitly stated that the analyses were blinded to subject group. Ten gave the time interval between stroke and endothelial function assessment (range <72 hours to three

months, median 26 days). The lacunar and cortical ischaemic stroke patients were not age-matched.

Characteristics of included patients and controls

All 16 papers defined lacunar ischaemic stroke as "appropriate neurological features and a recent small subcortical infarct on imaging consistent with the symptoms". Most papers excluded patients with carotid stenosis (except one[30]), five excluded cardiogenic sources of emboli,[32;33;36;38;41] and three excluded middle cerebral artery (MCA) stenosis[35;39;40] from the lacunar stroke group.

Thirteen studies had age-matched medically-confirmed normal controls; 5/13 also used imaging to exclude subjects with silent infarcts.[30;32;39;40;42] Three studies recruited age- and risk-factor matched controls (with long-standing hypertension and hypercholesterolaemia in two;[32;34] with hypertension only in one[43]). Five papers compared patients with lacunar ischaemic stroke to patients with cortical ischaemic stroke,[35;37;38;42;44] confirming the infarct subtype with CT or MRI. Four studies included patients with more than one lacunar infarct on CT,[30;37;40;45] MR,[40;45] or both.[37]

Assessment of endothelial function (Table 1)

Twelve papers assessed endothelial function in the cerebral circulation[30-32;34;37-42;44;45] and five papers the systemic circulation;[32;33;35;36;43] one assessed both.[32] Several techniques (Table 1) were used to assess cerebral endothelial function, including the vascular response to hypercapnia, infusion of acetazolamide or L-arginine, expressing the response as a percentage increase in mean arterial blood

velocity in the MCA or basilar artery. Change in blood oxygen level dependent signal during hypercapnia detected using functional MRI,[31] percent increase in regional cerebral blood flow in response to hypercapnia using stable Xenon CT[30] and "dynamic cerebral autoregulation" (the ability to restore cerebral blood flow following sudden changes in perfusion pressure)[38] were also used. Peripheral endothelial function was assessed with brachial artery flow-mediated dilatation, expressed as the percentage change in arterial diameter, in five papers;[32;33;35;36;43] one also assessed endothelium-independent flow-mediated dilatation using response to sublingual nitroglycerine.[43]

Endothelial function: Lacunar stroke vs. age-matched controls

Thirteen studies (n=534) compared lacunar ischaemic stroke to healthy age-matched controls, nine in the cerebral (n=360)[30-32;34;37;39-42] and four in the peripheral circulation (n=211).[32;33;35;36] Vascular reactivity was reduced in the cerebral circulation in lacunar stroke patients compared with age-matched healthy controls (8/9 studies, 324 patients, SMD -0.94, 95% confidence intervals (CI) -1.17, -0.70, p<0.00001, Figure 1) and in the forearm (4/4 studies, 211 patients, SMD -1.04, 95% CI -1.33, -0.75, p<0.00001, Figure 2). There was no significant heterogeneity between studies.

Lacunar ischaemic stroke vs. age+risk factor-matched controls

Four papers compared vascular reactivity in lacunar stroke patients with age+risk-factor-matched controls, two in the cerebral[32;34] and three in the peripheral circulation.[32;33;43] There was no significant difference in cerebrovascular reactivity (2/2 studies, 79 patients, SMD 0.08, 95% CI -0.36, 0.53, p=0.71, Figure 1) but

significantly impaired peripheral vascular reactivity (3/3 studies, 167 patients, SMD -0.94, 95% CI -1.26, -0.61, p<0.00001, Figure 2). There was no significant heterogeneity between studies.

Lacunar vs. cortical ischaemic stroke

Four of the 16 studies (n=127)[37;38;42;44] compared cerebral vascular reactivity in patients with lacunar stroke (n=68) to patients with cortical stroke (n=54), of which two studies contributed to more than one comparison (Figure 1). One study (n=117) compared peripheral vascular reactivity between lacunar (n=56) and cortical (n=61) stroke patients (Figure 2).[35] For the cerebral comparisons, we used the test results from the asymptomatic side of the brain, because vascular reactivity was reduced in ipsilateral vs. contralateral arteries in cortical stroke patients due to tissue damage resulting from the stroke (see Table 1). Although individual studies showed differences in endothelial function between lacunar and cortical stroke patients[35;37;38;42;44] the combined data showed no difference in vascular reactivity between lacunar and cortical stroke in either cerebral (SMD -0.29, 95% CI -0.69, 0.11, p=0.16) or in peripheral (SMD -0.23, 95% CI -0.55, 0.08, p=0.15) circulation. There was no significant heterogeneity between studies.

Lacunar ischaemic stroke with single vs. multiple silent lacunar infarcts

Four of the 16 papers[30;37;40;45] compared cerebral vascular reactivity in lacunar stroke patients with one single lacunar infarction (n=71) to those with additional multiple silent infarctions (n=71) on imaging (Figure 1). Patients with lacunar ischaemic stroke plus multiple silent lacunar infarcts had reduced cerebral vascular reactivity

compared to patients without silent lacunar infarcts on imaging (SMD -0.68, 95% CI -1.02, -0.34, p=0.0001), with no significant heterogeneity between studies.

Discussion

Endothelial dysfunction is common in patients with symptomatic large artery atheromatous disease and has also been postulated as a mechanism underlying the development of lacunar stroke. This systematic review suggests that lacunar ischaemic stroke is associated with impaired vascular reactivity compared with normal age-matched controls, but the association is less clear-cut when compared with controls matched for vascular risk factors or patients with cortical ischaemic stroke. One interpretation of this is that endothelial dysfunction may be a general response of the vascular system to the vascular risk factors that predispose to stroke,[9] or other circumstances associated with stroke such as secondary prevention medications, rather than being specific to small or large artery disease.

A previous systematic review identified associations between lacunar stroke and altered vascular reactivity, but did not perform a meta-analysis or make direct comparisons between lacunar stroke and patients with vascular risk factors or cortical stroke controls.[12] Endothelial function is altered in the presence of vascular risk factors[9] and by drugs used for risk factor reduction and stroke prevention.[10] Cortical stroke patients control for use of medications and presence of vascular risk factors so are the most valid comparison.[46] It is worth noting that the presence of an infarct in the brain was associated with reduced ipsilateral vascular reactivity, e.g. cortical stroke patients had reduced reactivity ipsilateral to the ischaemic cortical stroke. Hence any reduction in cerebral vascular reactivity in patients with multiple silent lacunar infarcts on imaging in both hemispheres (compared to patients with

only a single lacunar infarct) is unsurprising and is likely to be a consequence of more brain damage.

The strengths include following well-established guidance for conducting systematic reviews of observational and diagnostic data (www.equator-network.org) and Cochrane Stroke Group search advice. We used standard pre-specified criteria for study assessment. We carefully avoided duplicate data. Some studies provided more than one comparison but we were careful to avoid double counting the total number of subjects. In patients with stroke, we only used the cerebral vascular reactivity results from the asymptomatic side of the brain to avoid simply measuring the effects of brain damage resulting from the index stroke. We meta-analysed the data thereby effectively increasing sample size and precision.

The limitations include the small number of relevant studies, their small sample size, the varied and often poorly described diagnosis of lacunar stroke and the various and poorly standardised endothelial function tests used. In general, the papers gave little detail about how the diagnosis of lacunar stroke had been made clinically and/or with imaging, so inevitably there will be some “noise” due to imprecise diagnoses. However, given the relative lack of literature on this topic, we decided that it would be better to include studies which appeared to have included patients with symptomatic lacunar stroke as any attempt to exclude studies on the basis of their lacunar stroke diagnosis could have resulted in further bias. Studies which used suboptimal imaging, either insensitive or applied too late after the acute symptoms, may have confused up to 20% of lacunar strokes as cortical strokes and vice versa.[47] It was often unclear if the investigators were blind to study group; unblinding

may increase investigator bias. The endothelial function data were not adjusted for potential confounders such as blood pressure, diabetes, hypercholesterolaemia, smoking, prior stroke, white matter hyperintensities on imaging, old infarcts or haemorrhages on imaging, age or medication. Although studies generally matched with healthy controls for age, the lacunar and cortical stroke groups were not well age-matched. Despite many antihypertensive and stroke prevention medications being known to influence endothelial function, there was little information about current medications, most studies did not indicate if medications had been stopped prior to the study, and where this was mentioned, it was a very short time (e.g. 12 hours[35]) before the endothelial function studies. While some studies used hospital controls, recruitment procedures (source, mechanism) were unclear for many studies. Other limitations reflect the limited resources available for this review, e.g. we were unable to obtain translations for three papers which might have contained relevant data. The cerebral circulation studies used several different endothelial function tests.[15] However it is important to realise that the meta-analysis does not directly compare studies with each other, but rather the magnitude of association within each study with that in other studies. Therefore the grouping of apparently different methods of assessing endothelial function is more valid than attempting to combine data from different studies that used different methods in an individual patient data meta-analysis. There is also likely to be publication bias, meaning that the present analyses are over positive.

Is there a need for further research on endothelial function and lacunar stroke? The existing data do not exclude a specific association between endothelial dysfunction and lacunar stroke. Based on the modest difference in the cerebral circulation

between lacunar and cortical patients identified in this review and the large standard deviation (SD) of the endothelial function measurement methods, a future study would require a total sample size of 570 patients (half lacunar and half cortical) to confirm a difference in cerebral vascular reactivity of 22%, SD 20%, with 80% power at the $p<0.05$ level, particularly if there were to be any adjustment for even a few key potential confounding variables. If the SD could be reduced, e.g. by increasing the precision of the endothelial function measurements (although ±20% is biologically very plausible and any less would be unlikely), then the sample sizes would be smaller. On the other hand, a difference of 22% is optimistic, the differences in the present studies being nearer 6%, in which case a sample size of 752 would be required. The studies to date were much smaller than this. Numerous studies report plasma markers of endothelial function (e.g. asymmetric dimethylarginine[12;48]) and lacunar disease, but the identification and meta-analyses of these studies was beyond the remit of this review. There may be an association between angiotensin-converting enzyme insertion/deletion polymorphism (influencing endothelial function) and leukoaraiosis,[49] but the results of genetic association studies are awaited.

In addition to including a control group with a pathophysiologically different subtype of ischaemic stroke, future studies should ensure optimal clinical and imaging diagnosis of stroke subtype, provide clear descriptions of their recruitment and assessment methods, ensure adequate blinding of endothelial assessments, have appropriate controls drawn from a relevant and comparative population, record medications, try to balance study groups for medications, preferably discontinue vasoactive drugs prior to study, adjust for differences in vascular risk factors, and match for age. The peripheral circulation provides a valuable method of examining

systemic endothelial dysfunction outside the territory affected by the recent stroke. Cerebral small vessel disease may be a systemic small vessel problem affecting multiple organs,[8;50] in the same way that large artery atheroma is rarely a disease of only one large artery, and therefore it is legitimate and necessary to study small vessel disease in multiple organs, not just the brain.

References

1. Hachinski V. Stroke and vascular cognitive impairment: a transdisciplinary, translational and transactional approach. *Stroke* 2007;**38**:1396-403.

2. Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet* 1991;**337**:1521-6.

3. Vermeer SE, Longstreth WT, Jr., Koudstaal PJ. Silent brain infarcts: a systematic review. *Lancet Neurol* 2007;**6**:611-9.

4. Jackson C, Sudlow C. Comparing risks of death and recurrent vascular events between lacunar and non-lacunar infarction. *Brain* 2005;**128**:2507-17.

5. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MMB. Silent brain infarcts and the risk of dementia and cognitive decline. *N Engl J Med* 2003;**348**:1215-22.

6. Hachinski V. World Stroke Day 2008: "Little strokes, big trouble". *Stroke* 2008;**39**:2407-20.

7. Hassan A, Hunt BJ, O'Sullivan M, Parmar K, Bamford JM, Briley D, Brown MM, Thomas DJ, Markus HS. Markers of endothelial dysfunction in lacunar infarction and ischaemic leukoaraiosis. *Brain* 2003;**126**:424-32.

8. Thompson CS, Hakim AM. Living beyond our physiological means. Small vessel disease of the brain is an expression of a systemic failure in arteriolar function: a unifying hypothesis. *Stroke* 2009;**40**:e322-e330.

9. Girouard H, Iadecola C. Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. *J Appl Physiol* 2006;**100**:328-35.

10. Webb DJ. The pharmacology of human blood vessels in vivo. *J Vasc Res* 1995;**32**:2-15.

11. Newby DE, McLeod AL, Uren NG, Flint L, Ludlam CA, Webb DJ, Fox KA, Boon NA. Impaired coronary tissue plasminogen activator release is associated with coronary atherosclerosis and cigarette smoking: direct link between endothelial dysfunction and atherothrombosis. *Circulation* 2001;**103**:1936-41.

12. Knottnerus ILH, Ten Cate H, Lodder J, Kessels F, van Oostenbrugge RJ. Endothelial dysfunction in lacunar stroke: a systematic review. *Cerebrovasc Dis* 2009;**27**:519-26.

13. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, for the STROBE initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;**370**:1453-7.

14. Whiting P, Rutjes AWS, Reitsma JB, Bossuyt PMM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003;**3**:25.

15. Tousoulis D, Antoniades C, Stefanadis C. Evaluating endothelial function in humans: a guide to invasive and non-invasive techniques. *Heart* 2005;**91**:553-8.

16. Shutov AA, Baidina TV, Agafonov AV, Siutkina OV, Gaidash GV. [Endothelium dysfunction in patients with ischemic stroke]. *Zh Nevrol Psikhiatr im S S Korsakova* 2005;**Suppl 14**:42-5.

17. Gaspar L, Stvrtinova V, Gavornik P, Strbova L, Porubec V. [Changes in forearm microcirculation in patients with focal cerebral ischemia]. *Bratisl Lek Listy* 1998;**99**:122-3.

18. Panczel G, Bonoczk P, Nagy Z. [Impairment of vasoreactivity in brainstem and hemispheral small vessel disease: comparative study]. *Ideggyogy Sz* 2002;**55**:95-101.

19. Birns J, Jarosz J, Markus HS, Kalra L. Cerebrovascular reactivity and dynamic autoregulation in ischaemic subcortical white matter disease. *J Neurol Neurosurg Psychiatry* 2009.

20. Gommer ED, Staals J, van Oostenbrugge RJ, Lodder J, Mess WH, Reulen JP. Dynamic cerebral autoregulation and cerebrovascular reactivity: a comparative study in lacunar infarct patients. *Physiol Meas* 2008;**29**:1293-303.

21. Walters M, Muir S, Shah I, Lees K. Effect of perindopril on cerebral vasomotor reactivity in patients with lacunar infarction. *Stroke* 2004;**35**:1899-902.

22. Sterzer P, Meintzschel F, Rosler A, Lanfermann H, Steinmetz H, Sitzer M. Pravastatin improves cerebral vasomotor reactivity in patients with subcortical small-vessel disease. *Stroke* 2001;**32**:2817-20.

23. Lee KO, Lee KY, Lee SY, Ahn CW, Park JS. Lacunar infarction in type 2 diabetes is associated with an elevated intracranial arterial pulsatility index. *Yonsei Med J* 2007;**48**:802-6.

24. Imaizumi T, Chiba M, Honma T, Yoshikawa J, Niwa J. Cerebral blood flow before and after treatment with amlodipine in elderly patients with lacunar infarction. *Clin Drug Investig* 2004;**24**:765-9.

25. Terborg C, Gora F, Weiller C, Rother J. Reduced vasomotor reactivity in cerebral microangiopathy: a study with near-infrared spectroscopy and transcranial Doppler sonography. *Stroke* 2000;**31**:924-9.

26. Zvan B, Zaletel M, Pogacnik T, Kiauta T. Testing of cerebral endothelium function with L-arginine after stroke. *Int Angiol* 2002;**21**:256-9.

27. Pasqualini L, Marchesi S, Vaudo G, Siepi D, Angeli F, Paris L, Schillaci G, Mannarino E. Association between endothelial dysfunction and major cardiovascular events in peripheral arterial disease. *Vasa* 2003;**32**:139-43.

28. De Reuck J, Decoo D, Hasenbroekx MC, Lamont B, Santens P, Goethals P, Strijckmans K, Lemahieu I. Acetazolamide vasoreactivity in vascular dementia: a positron emission tomographic study. *Eur Neurol* 1999;**41**:31-6.

29. Mindach M. [Correlation between low cerebral flow velocities determined by transcranial ultrasound and lacunar cerebral infarction]. *Ultraschall Med* 2001;**22**:274-8.

30. Mochizuki Y, Oishi M, Takasu T. Cerebral blood flow in single and multiple lacunar infarctions. *Stroke* 1997;**28**:1458-60.

31. Hund-Georgiadis M, Zysset S, Naganawa S, Norris DG, Yves von Cramon D. Determination of cerebrovascular reactivity by means of fMRI signal changes in cerebral microangiopathy: a correlation with morphological abnormalities. *Cerebrovasc Dis* 2003;**16**:158-65.

32. Pretnar-Oblak J, Sabovic M, Sebestjen M, Pogacnik T, Zaletel M. Influence of atorvastatin treatment on L-arginine cerebrovascular reactivity and flow-mediated dilatation in patients with lacunar infarctions. *Stroke* 2006;**37**:2540-5.

33. Pretnar-Oblak J, Sabovic M, Pogacnik T, Sebestjen M, Zaletel M. Flow-mediated dilatation and intima-media thickness in patients with lacunar infarctions. *Acta Neurol Scand* 2006;**113**:273-7.

34. Pretnar-Oblak J, Zaletel M, Zvan B, Sabovic M, Pogacnik T. Cerebrovascular reactivity to L-arginine in patients with lacunar infarctions. *Cerebrovasc Dis* 2006;**21**:180-6.

35. Chen PL, Wang PY, Sheu WH, Chen YT, Ho YP, Hu HH, Hsu HY. Changes of brachial flow-mediated vasodilation in different ischemic stroke subtypes. *Neurology* 2006;**67**:1056-8.

36. Lavallee PC, Bonnin P, Labreuche J, Amarenco P, Levy B. Flow-mediated vasodilatation of carotid and brachial arteries in healthy subjects and in lacunar stroke patients. *Ultrasound Med Biol* 2006;**32**:1165-9.

37. Cupini LM, Diomedi M, Placidi F, Silvestrini M, Giacomini P. Cerebrovascular reactivity and subcortical infarctions. *Arch Neurol* 2001;**58**:577-81.

38. Immink RV, van Montfrans GA, Stam J, Karemaker JM, Diamant M, van Lieshout JJ. Dynamic cerebral autoregulation in acute lacunar and middle cerebral artery territory ischemic stroke. *Stroke* 2005;**36**:2595-600.

39. de Leeuw FE, van Huffelen A, Kappelle J. Cerebrovascular reactivity in patients with a recent lacunar infarction. *J Neurol* 2003;**250**:232-3.

40. Molina C, Sabin JA, Montaner J, Rovira A, Abilleira S, Codina A. Impaired cerebrovascular reactivity as a risk marker for first-ever lacunar infarction: a case-control study. *Stroke* 1999;**30**:2296-301.

41. Panczel G, Bonoczk P, Voko Z, Spiegel D, Nagy Z. Impaired vasoreactivity of the basilar artery system in patients with brainstem lacunar infarcts. *Cerebrovasc Dis* 1999;**9**:218-23.

42. Maeda H, Matsumoto M, Handa N, Hougaku H, Ogawa S, Itoh T, Tsukamoto Y, Kamada T. Reactivity of cerebral blood flow to carbon dioxide in various types of ischemic cerebrovascular disease: evaluation by the transcranial Doppler method. *Stroke* 1993;**24**:670-5.

43. Kim JS, Lee HS, Park HY, Kim SS, Kang HG, Kim NH, Park JS, Kim Y. Endothelial function in lacunar infarction: a comparison of lacunar infarction, cerebral atherosclerosis and control group. *Cerebrovasc Dis* 2009;**28**:166-70.

44. Gur AY, Gucuyener D, Uzuner N, Gilutz Y, Ozdemir G, Korczyn AD, Bornstein NM. Cerebral vasomotor reactivity of patients with acute ischemic stroke: cortical versus subcortical infarcts: an Israeli-Turkish collaborative study. *J Neurol Sci* 2007;**257**:121-5.

45. Chamorro A, Saiz A, Vila N, Ascaso C, Blanc R, Alday M, Pujol J. Contribution of arterial blood pressure to the clinical expression of lacunar infarction. *Stroke* 1996;**27**:388-92.

46. Jackson CA, Sudlow CLM. Are lacunar strokes really different? A systematic review of differences in risk factor profiles between lacunar and non-lacunar infarcts. *Stroke* 2005;**36**:891-904.

47. Potter G, Doubal F, Jackson C, Sudlow C, Dennis M, Wardlaw J. Associations of clinical stroke misclassification ('clinical-imaging dissociation') in acute ischemic stroke. *Cerebrovasc Dis* 2009;in press.

48. Rufa A, Blardi P, De Lalla A, Cevenini G, De Stefano N, Zicari E, Auteri A, Federico A, Dotti MT. Plasma levels of asymmetric dimethylarginine in cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy. *Cerebrovasc Dis* 2008;**26**:636-40.

49. Paternoster L, Chen W, Sudlow C. Genetic determinants of white matter hyperintensities on brain scans. *Stroke* 2009;**40**:2020-6.

50. Luijckx G-J, Boiten J, van Kroonenburgh M, Kitslaar P, Kurvers H, Daemen M, Leunissen K, Beintema M, Lodder J. Systemic small-vessel disease is not exclusively related to lacunar stroke. A pilot study. *J Stroke Cerebrovasc Dis* 1998;**7**:52-7.

Table 1. Dynamic endothelial function in the a) cerebral and b) peripheral circulation. Included studies evaluating dynamic cerebral and peripheral endothelial function in patients with lacunar stroke and controls.

a) Cerebral circulation

Study	Year	Lacunar subject details	Lacunar number	Control number	Clinical classification	Brain imaging modality	Endothelial function method	Endothelial function results	
								Lacunar	Controls
Single lacunar stroke vs. normal age-matched controls				**Age-matched**					
Pretnar-Oblak (a)[32]	2006	Lacunar stroke with hypercholesterolaemia	18 (61.1±7.6)	19 (59.2±7.1)	TOAST	CT	% increase in mean MCA blood flow velocity on TCD after L-arginine	13.1±8.4	21.3±10.9
Pretnar-Oblak (c)[34]	2006	Lacunar stroke	20 (60.9±6.2)	21 (59.5±7.3)	Not stated	CT	% increase in mean MCA blood flow velocity on TCD after L-arginine	13.4±9.1	20.5±9.9
Mochizuki[30]	1997	Single lacunar stroke	15 (63.5)	16 (58.4)	Not stated	CT	stable xenon CT:	7.3± 6.0	14.3±11.5
							absolute % increase in rCBF of white matter after acetazolamide	41.9%	60.3% ↑
Molina[40]	1999	First-ever lacunar stroke	46 (56.6±13.4)	46 (58.3±12)	Based on imaging	MRI	% increase in mean MCA flow velocity on TCD after acetazolamide	50±12.7	65.2±12.4
Panczel[41]	1999	Brainstem lacunar stroke	20 (62.2±13.9)	10 (64.4±4.3)	Not stated	CT/MRI	increase in mean BA flow velocity on TCD after acetazolamide (%)	47.3±21.9	53.6±20.2
							Response to hyper-capnia cm/sec/kPA	3.4± 5.0	10.1± 4.9
de Leeuw[39]	2003	Single lacunar stroke	12 (58.2±16.8)	12 (52±12.1)	Not stated	CT/MRI	% increase in mean MCA blood flow velocity on TCD per mmHg CO_2 increase	3.0±1.3	4.8±1.9

Study	Year	Lacunar subject details	Lacunar number	Control number	Clinical classification	Brain imaging modality	Endothelial function method	Endothelial function results	
								Lacunar	Controls
							CO_2		
Maeda[42]	1993	Lacunar stroke	20 (59.6±6.8)	25 (57.3±6.5)	NINDS	CT	Mean spatial Doppler frequency=A exp(**k** Pet CO_2) hypercapnia	0.028±0.004	0.033±0.005
Cupini[37)]	2001	Lacunar stroke	14 (61.4±9.2)	15 (57.66±12.7)	Not stated	CT/MRI	BHI: ∆MFV/Baseline MFV x 100/s	1.36± 0.39	1.60±0.40
Hund-Georgiadis[31]	2003	Lacunar infarction and leukoar-aiaosis	5 (61.8)	6 (57)	Not stated	MRI	Measured using fMRI: BOLD signal volume decrease (cm^3) change normalised to ET- CO_2	290±190	480±160
Immink[38]	2005	Lacunar stroke	10 (63±3)	10 (57±2)	Not stated	CT/MRI	delay (in seconds) of MCA Vmean counter-regulation during changes in MAP increments in seconds*	Passively followed MAP, i.e. no latency of response	5.3±0.5
Single lacunar stroke vs. age and risk-factor matched controls				**Age and risk-factor matched**					
Pretnar-Oblak (a)[32]	2006	Lacunar stroke with hyperchol-esterolaemia	18 (61.1±7.6)	20 (62.7±5.3)	TOAST	CT	% increase in mean blood velocity on TCD after L-arginine	13.1 ±8.4	13.5 ±8.3
Pretnar-Oblak (c)[34]	2006	Lacunar infarction	20 (60.9 ±7.3)	21 (61.0±6.2)	Not stated	CT	% increase in mean blood velocity on TCD after L-arginine	13.4 ±9.1	11.5 ±8.9
Single lacunar vs. cortical ischaemic stroke				**Cortical ischaemic stroke**					
Gur[44]	2007	Lacunar infarction	24 (60.5±13.71)	23 (72±13.7)	Not stated	CT/MRI	% increase in mean blood velocity after acetazolamide Ig IV	25.6 ±12.2 (ipsilateral) 19.1 ±19.5	12.2 ±15.9 (ipsilateral) 30.6 ±28.1

Study	Year	Lacunar subject details	Lacunar number	Control number	Clinical classification	Brain imaging modality	Endothelial function method	Endothelial function results	
								Lacunar	Controls
								(contralateral)	(contralateral)
Maeda[42]	1993	Lacunar infarction	20 (59.6±6.8)	8 (53.5±9.5)	NINDS	CT/MRI	Mean spatial Doppler frequency= A exp(**k** Pet CO_2) response to CO_2	0.027±0.004 (ipsilateral) 0.028±0.004 (contralateral)	0.027±0.004 ipsilateral) 0.031±0.008 (contralateral)
Cupini[37]	2001	Lacunar infarction	14 (61.4±9.2)	13 (53.9±11.8)	Not stated	CT/MRI	BHI: ΔMFV/Baseline MFV x 100/s	1.36±0.39 (ipsilateral) 1.33±0.36 (contralateral)	1.45±0.51 (contralateral) 1.24±0.51 (ipsilateral)
Immink[38]	2005	Lacunar infarction	10 (63±3)	10 (59±5)	Not stated	CT/MRI	dCA: delay of MCA Vmean counter-regulation during changes in MAP increments in seconds	Passively followed MAP	Not detectable (ipsilateral) 4.6±0.7 (↑) (contralateral)
Single vs. multiple lacunar infarcts on imaging			**Single**	**Multiple**					
Chamorro[45]	1996	Lac stroke + single or multiple infarcts on imaging	21 (NA)	22 (NA)	Stroke Data Bank	MRI	% increase in mean MCA flow velocity after acetazolamide	29.6±28.2 (ipsilateral) 35.0±21.7 (contralateral)	35.5±17.6 (ipsilateral) 49.1±31.2 (contralateral)
Molina[40]	1999	Lac stroke + single or multiple infarcts on imaging	26 (NA)	20 (NA)	Based on imaging	MRI	% increase in mean MCA flow velocity after acetazolamide (contralateral only)	46.38±12.6	54.83±11.58

Study	Year	Lacunar subject details	Lacunar number	Control number	Clinical classification	Brain imaging modality	Endothelial function method	Endothelial function results	
								Lacunar	Controls
Mochizuki[30]	1997	Lac stroke + single or multiple infarcts on imaging	10 (61.6)	15 (63.5)	Not stated	CT	stable xenon CT method increase in rCBF of white matter after acetazolamide (contralat only)		
							absolute increase:	5.0±3.4	7.3±6.0
							% increase :	34.2%	41.9%
Cupini[37]	2001	Lac stroke + single or multiple infarcts on imaging	14 (60.5±10.5)	14 (61.4±9.2)	Not stated	CT/MRI	BHI: ΔMFV/Baseline MFV x 100/s Breath holding	0.97±0.42 (ipsilateral) 0.90±0.36 (contralateral)	1.33±0.36 (ipsilateral) 1.36±0.39 (contralateral)

b) Peripheral circulation

Study	Year	Lacunar subject details	Lacunar number (age)	Control number (age)	Clinical classification	Brain imaging modality	Endothelial function method	Endothelial function results	
								Lacunar	Controls
Single lacunar stroke vs. normal age-matched controls				**Age-matched controls**					
Pretnar-Oblak (a)[32]	2006	Lacunar stroke with hypercholesterol-aemia	18 (61.1±7.6)	19 (59.2±7.1)	TOAST	CT	Flow mediated dilatation: % increase in brachial artery diameter after cuff inflation	0.06±4.9	8.1±6.0
Pretnar-Oblak (b)[33]	2006	Lacunar stroke	20 (60.9±7.3)	21 (59.5±7.3)	Scan based	CT	Flow mediated dilatation: % increase in brachial artery diameter, after cuff inflation+deflation	0.4±5.0	7.9 ± 6.0 %
Chen[35]	2006	Lacunar stroke	56(67.7±10.2)[§]	40 (66.7±8.3)	TOAST	CT/MRI	Flow mediated dilatation: % increase in brachial artery diameter after cuff inflation+deflation	4.3±6.1	8.8±6.0
Lavallee[36]	2006	Lacunar stroke	17 (61±21)	20 (60±25)	Not stated	Not stated	Flow mediated dilatation: % increase in brachial artery diameter after cuff inflation+deflation	4.2±1.1	6.7±2.5
Lavallee[36]	2006	Lacunar stroke	17 (61±21)	20 (60±25)	Not stated	Not stated	Flow mediated dilatation: % increase in carotid artery diameter after CO_2	4.0±1.	11.4±2.7
Single lacunar stroke vs. age+risk-factor matched controls				**Age +risk-factor matched controls**					
Pretnar-	2006	Lacunar stroke	18 (61.1±7.6)	20 (62.7±5.3)	TOAST	CT	Flow mediated	0.06±4.9	3.1±4.8

Study	Year	Lacunar subject details	Lacunar number (age)	Control number (age)	Clinical classification	Brain imaging modality	Endothelial function method	Endothelial function results	
								Lacunar	Controls
Oblak (a)[32]		with hypercholesterol-aemia					dilatation: % increase in brachial artery diameter after cuff inflation+deflation		
Pretnar-Oblak (b)[33]	2006	Lacunar stroke	20 (60.9±7.3)	20 (61.0±6.2)	Scan based	CT	Flow mediated dilatation: % increase in brachial artery diameter after cuff inflation+deflation	0.4±5.0	3.8±4.8
Kim[43]	2009	Lacunar ischaemic stroke	45 (60±7)	44 (59±8)	"typical clinical features + MRI"	MR	Flow mediated dilatation: % increase in brachial artery diameter after cuff inflation+deflation	6.6±4.5 [14.3±4.9] +	12.2±4.6 [13.8±4.9] +
Single lacunar vs. cortical ischaemic stroke				**Cortical ischaemic stroke**					
Chen[35]	2006	Lacunar stroke vs. large artery atheroma stroke	56(67.6±10.2)§	40 (67.8±8.2)	TOAST	CT/MRI	Flow mediated dilatation: % increase in brachial artery diameter after cuff inflation+deflation	4.3±6.1	5.7±5.4
Chen[35] "Chen 2" in Fig 2	2006	Lacunar stroke vs. cardio embolic stroke	56(67.6±10.2)§	21 (67.6±9.2)	TOAST	CT/MRI	Flow mediated dilatation: % increase in brachial artery diameter after cuff inflation+deflation	4.3±6.1	5.6±5.0

*for back to back BP changes; +for flow mediated dilatation in response to sublingual nitroglycerine (endothelium independent dilatation); § note that there are 56 patients with lacunar stroke in total in Chen 2006, not 56 x 3. BA, basilar artery; BHI, breath holding index; BOLD, blood oxygen level dependent; CT, computerised tomography; fMRI, functional magnetic resonance imaging; MAP, mean arterial pressure; MCA, middle cerebral artery; MFV, mean flow velocity; MRI, magnetic resonance imaging; rCBF, regional cerebral blood flow; TCD, transcranial Doppler.

Figure 1 Forest plot showing standardised mean difference (SMD) in cerebrovascular reactivity in patients with lacunar ischaemic stroke vs. age-matched, age+risk factor matched and cortical ischaemic stroke controls and in patients with lacunar ischaemic stroke with vs. without multiple silent lacunar infarcts. Squares represent the ratio of the lacunar response divided by the control response with the solid black lines representing the 95% CI. The diamond represents the summary result.

Figure 2 Forest plot showing standardised mean difference (SMD) in peripheral endothelial function in patients with lacunar ischaemic stroke vs. age-matched, age and risk factor matched and cortical ischaemic stroke controls. See Figure 1 for legend. Note that Chen[35] provided data for cortical stroke as carotid stenosis (a) and cardioembolic (b) mechanisms separately, not for "all cortical stroke", hence there are two entries for Chen (a and b) under "lacunar vs cortical" – thus the 56 patients with lacunar stroke appear twice.

Figure1

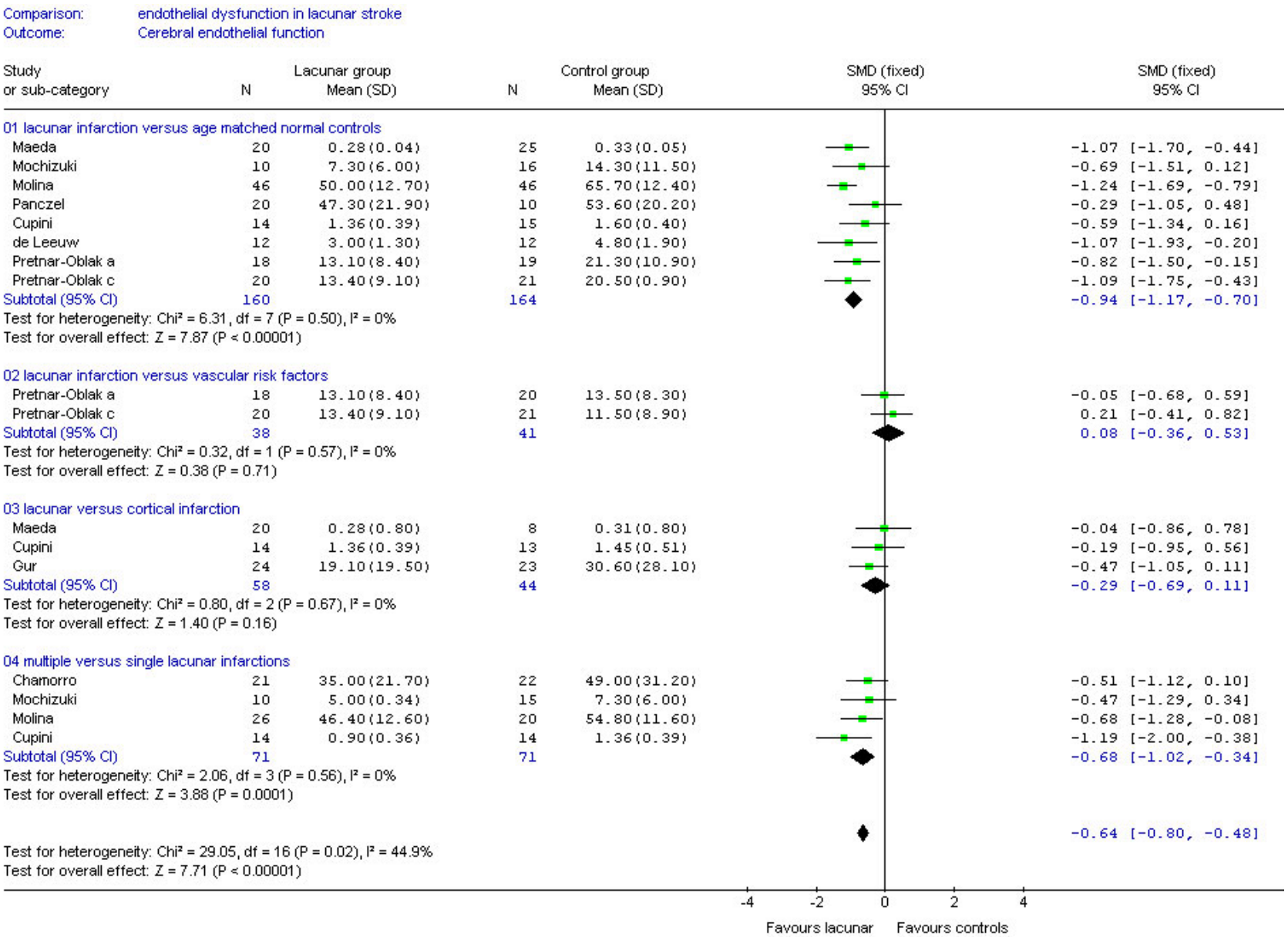
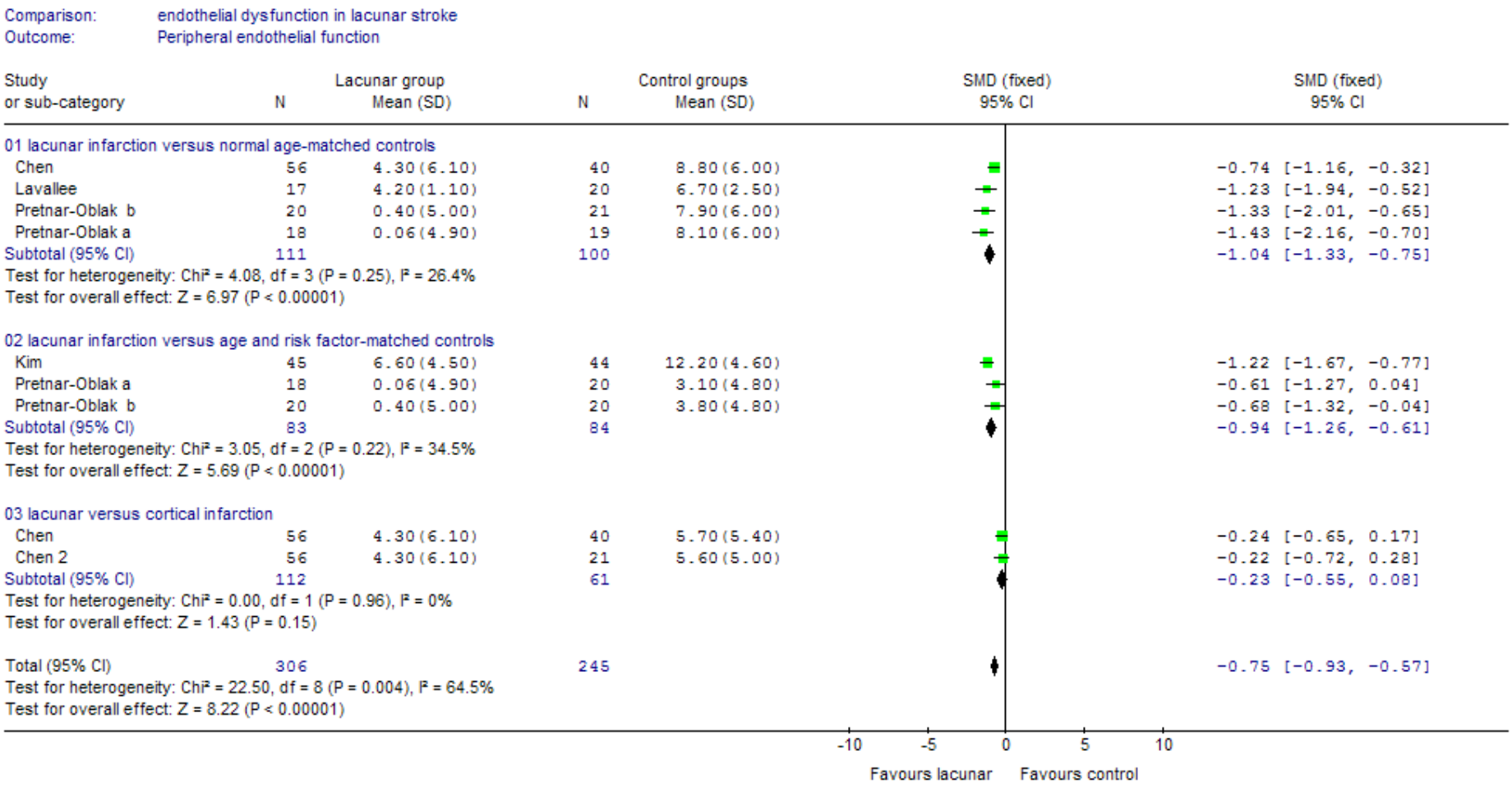

Comparison: endothelial dysfunction in lacunar stroke
Outcome: Cerebral endothelial function
Study or sub-category | Lacunar group N Mean (SD) | Control group N Mean (SD) | SMD (fixed) 95% CI | SMD (fixed) 95% CI
01 lacunar infarction versus age matched normal controls
Maeda 20 0.28(0.04) 25 0.33(0.05) -1.07 [-1.70, -0.44]
Mochizuki 10 7.30(6.00) 16 14.30(11.50) -0.69 [-1.51, 0.12]
Molina 46 50.00(12.70) 46 65.70(12.40) -1.24 [-1.69, -0.79]
Panczel 20 47.30(21.90) 10 53.60(20.20) -0.29 [-1.05, 0.48]
Cupini 14 1.36(0.39) 15 1.60(0.40) -0.59 [-1.34, 0.16]
de Leeuw 12 3.00(1.30) 12 4.80(1.90) -1.07 [-1.93, -0.20]
Pretnar-Oblak a 18 13.10(8.40) 19 21.30(10.90) -0.82 [-1.50, -0.15]
Pretnar-Oblak c 20 13.40(9.10) 21 20.50(0.90) -1.09 [-1.75, -0.43]
Subtotal (95% CI) 160 164 -0.94 [-1.17, -0.70]
Test for heterogeneity: Chi² = 6.31, df = 7 (P = 0.50), I² = 0%
Test for overall effect: Z = 7.87 (P < 0.00001)
02 lacunar infarction versus vascular risk factors
Pretnar-Oblak a 18 13.10(8.40) 20 13.50(8.30) -0.05 [-0.68, 0.59]
Pretnar-Oblak c 20 13.40(9.10) 21 11.50(8.90) 0.21 [-0.41, 0.82]
Subtotal (95% CI) 38 41 0.08 [-0.36, 0.53]
Test for heterogeneity: Chi² = 0.32, df = 1 (P = 0.57), I² = 0%
Test for overall effect: Z = 0.38 (P = 0.71)
03 lacunar versus cortical infarction
Maeda 20 0.28(0.80) 8 0.31(0.80) -0.04 [-0.86, 0.78]
Cupini 14 1.36(0.39) 13 1.45(0.51) -0.19 [-0.95, 0.56]
Gur 24 19.10(19.50) 23 30.60(28.10) -0.47 [-1.05, 0.11]
Subtotal (95% CI) 58 44 -0.29 [-0.69, 0.11]
Test for heterogeneity: Chi² = 0.80, df = 2 (P = 0.67), I² = 0%
Test for overall effect: Z = 1.40 (P = 0.16)
04 multiple versus single lacunar infarctions
Chamorro 21 35.00(21.70) 22 49.00(31.20) -0.51 [-1.12, 0.10]
Mochizuki 10 5.00(0.34) 15 7.30(6.00) -0.47 [-1.29, 0.34]
Molina 26 46.40(12.60) 20 54.80(11.60) -0.68 [-1.28, -0.08]
Cupini 14 0.90(0.36) 14 1.36(0.39) -1.19 [-2.00, -0.38]
Subtotal (95% CI) 71 71 -0.68 [-1.02, -0.34]
Test for heterogeneity: Chi² = 2.06, df = 3 (P = 0.56), I² = 0%
Test for overall effect: Z = 3.88 (P = 0.0001)
-0.64 [-0.80, -0.48]
Test for heterogeneity: Chi² = 29.05, df = 16 (P = 0.02), I² = 44.9%
Test for overall effect: Z = 7.71 (P < 0.00001)
-4
-2
0
2
4
Favours lacunar
Favours controls

Figure 2


Comparison: endothelial dysfunction in lacunar stroke
Outcome: Peripheral endothelial function
Study or sub-category
Lacunar group N Mean (SD)
Control groups N Mean (SD)
SMD (fixed) 95% CI
SMD (fixed) 95% CI
01 lacunar infarction versus normal age-matched controls
Chen 56 4.30(6.10) 40 8.80(6.00) -0.74 [-1.16, -0.32]
Lavallee 17 4.20(1.10) 20 6.70(2.50) -1.23 [-1.94, -0.52]
Pretnar-Oblak b 20 0.40(5.00) 21 7.90(6.00) -1.33 [-2.01, -0.65]
Pretnar-Oblak a 18 0.06(4.90) 19 8.10(6.00) -1.43 [-2.16, -0.70]
Subtotal (95% CI) 111 100 -1.04 [-1.33, -0.75]
Test for heterogeneity: Chi² = 4.08, df = 3 (P = 0.25), I² = 26.4%
Test for overall effect: Z = 6.97 (P < 0.00001)
02 lacunar infarction versus age and risk factor-matched controls
Kim 45 6.60(4.50) 44 12.20(4.60) -1.22 [-1.67, -0.77]
Pretnar-Oblak a 18 0.06(4.90) 20 3.10(4.80) -0.61 [-1.27, 0.04]
Pretnar-Oblak b 20 0.40(5.00) 20 3.80(4.80) -0.68 [-1.32, -0.04]
Subtotal (95% CI) 83 84 -0.94 [-1.26, -0.61]
Test for heterogeneity: Chi² = 3.05, df = 2 (P = 0.22), I² = 34.5%
Test for overall effect: Z = 5.69 (P < 0.00001)
03 lacunar versus cortical infarction
Chen 56 4.30(6.10) 40 5.70(5.40) -0.24 [-0.65, 0.17]
Chen 2 56 4.30(6.10) 21 5.60(5.00) -0.22 [-0.72, 0.28]
Subtotal (95% CI) 112 61 -0.23 [-0.55, 0.08]
Test for heterogeneity: Chi² = 0.00, df = 1 (P = 0.96), I² = 0%
Test for overall effect: Z = 1.43 (P = 0.15)
Total (95% CI) 306 245 -0.75 [-0.93, -0.57]
Test for heterogeneity: Chi² = 22.50, df = 8 (P = 0.004), I² = 64.5%
Test for overall effect: Z = 8.22 (P < 0.00001)
-10
-5
0
5
10
Favours lacunar
Favours control

Appendix 1: Search Strategy

1. brain ischemia/ or brain infarction/ or brain stem infarctions/ or cerebral infarction/ or hypoxia-ischemia, brain/ or stroke/

2. (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.

3. ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.

4. 1 or 2 or 3

5. (lacun$ or small vessel$ or small infarct$ or microinfarct$ or subcortical lesion$ or subcortical infarct$ or microvascular$ or microcirculation$).tw.

6. 4 and 5

7. blood-brain barrier/ or endothel$, vascular/ or tunica intima/ or microcirculation/

8. (endotheli$ adj5 (function$ or dysfunction$ or impairment$)).tw.

9. ((vascular or capillary) adj5 endotheli$).tw.

10. (endotheli$ adj5 (contraction or relaxation)).tw.

11. vascular tone/ or arterial stiffness.mp. [mp=title, original title, abstract, name of substance word, subject heading word]

12. (vascul$ tone or neurovasc$ coupl$ or arterial stiff$ or vascul$ remodel$ or cerebrovascular reactiv$ or cerebral autoregulation).tw.

13. (Flow mediated adj3 (dilat$ or vasodilat$)).tw.

14. exp Ultrasonography, Doppler, Transcranial/

15. pulse wave analysis.tw.

16. strain gauge plethysmography.tw.

17. (brachial artery or radial artery or popiteal artery or posterior tibial artery).tw.

18. or/7-17

19. 6 and 18

20. limit 19 to yr="1995 - 2008"

21. limit 19 to humans

22. limit 21 to humans

23. from 22 keep 1-376

24. (strain gauge plethysmography or venous occlusion plethysmography).tw.

25. forearm blood flow.tw.

26. (dorsal hand vein technique or aellig technique).tw.

27. stimulated tPA release.tw.

28. or/24-27

29. 18 or 28

30. 6 and 29