Three-Dimensional MRI Analysis of Individual Volume of Lacunes in CADASIL

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Background and Purpose—Three-dimensional MRI segmentation may be useful to better understand the physiopathology of lacunar infarctions. Using this technique, the distribution of lacunar infarctions volumes has been recently reported in patients with cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Whether the volume of each lacune (individual lacunar volume [ILV]) is associated with the patients’ other MRI lesions or vascular risk factors has never been investigated. The purpose of this study was to study the impact of age, vascular risk factors, and MRI markers on the ILV in a large cohort of patients with CADASIL.

Methods—Of 113 patients with CADASIL, 1568 lacunes were detected and ILV was estimated after automatic segmentation on 3-dimensional T1-weighted imaging. Relationships between ILV and age, blood pressure, cholesterol, diabetes, white matter hyperintensities load, number of cerebral microbleeds, apparent diffusion coefficient, brain parenchymal fraction, and mean and median of distribution of lacunes volumes at the patient level were investigated. We used random effect models to take into account intraindividual correlations.

Results—The ILV varied from 4.28 to 1619 mm³. ILV was not significantly correlated with age, vascular risk factors, or different MRI markers (white matter hyperintensity volume, cerebral microbleed number, mean apparent diffusion coefficient or brain parenchymal fraction). In contrast, ILV was positively correlated with the patients’ mean and median of lacunar volume distribution ($P=0.0001$).

Conclusions—These results suggest that the ILV is not related to the associated cerebral lesions or to vascular risk factors in CADASIL, but that an individual predisposition may explain predominating small or predominating large lacunes among patients. Local anatomic factors or genetic factors may be involved in these variations.

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Key Words: CADASIL ■ cerebral lacunes ■ MRI

Deschambre and Durand-Fardel were the first authors to describe a “lacune” as an infarction related to the occlusion of perforating arteries with a diameter $<$300 μm.$^{1,2}$ Diagnosis of lacunar infarction is classically based on both the location and size of the lesion. In the literature, the upper limit of the size for a lacune is most often recorded in terms of diameter set at 15 mm.$^{3}$ This limit appears questionable because Fisher called “giant lacunes” ischemic lesions greater than 10 mm in diameter, whereas other authors have debated this artificial and nonvalidated cutoff.$^{4,5}$ Whether the volume of lacunes is comparable in all patients with cerebral small vessel disease (SVD) and if it is related to the severity of other ischemic tissue lesions or vascular risk factors or to the shrinking of cerebral tissue due to atrophy has never been investigated. Such questions remained unanswered possibly because of both technical limitations in pathological and imaging studies and the common association of atherosclerosis of larger arterial trunks, particularly of medium-sized arteries$^{6,7}$ in cases with SVD and “lacunar infarctions.”

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is considered as a unique model to investigate SVD involving perforating arteries of diameter less than 500 μm.$^{8}$ Recent developments of imaging techniques can be used to obtain 3-dimensional segmentation of circumscribed cerebral regions and in vivo calculation of their volume. In a recent study of patients with CADASIL, the shape and volume of individual lacunar lesions was analyzed using 3-dimensional MRI segmentation of millimetric T1-weighted images.$^9$ The estimated volume of the 109 segmented lacunes (ie, the individual lacunar volume

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(ILV) ranged between 10 and 1146 mm$^3$. Interestingly, the mean ILV was found to be higher in the white matter than in the gray matter. These data suggested that cerebral location of lacunar infarcts may influence their volume. However, the limited sample size in this study (10 patients) precluded more definitive conclusions. In addition, the potential influence of vascular risk factors and other brain abnormality MRI markers (brain atrophy and other subcortical MRI markers) on the ILV was not analyzed.

The aim of the current study was to investigate the potential impact of age and different vascular risk factors on the ILV and to determine the associations between ILV and various MRI markers of SVD at the patient’s level (total lacunar volume, extent of white matter hyperintensities [WMH], number of cerebral microhemorrhages, diffusion change, and amount of cerebral atrophy) in a large cohort of patients with CADASIL.

**Methods**

**Subjects**

Subjects were drawn from an ongoing 2-center prospective cohort study of patients with CADASIL. Subjects were recruited among consecutive patients with CADASIL aged at least 18 years old, who consulted at Lariboisere (Paris, France) or Ludwig-Maximilians-Universität (Munich, Germany) hospitals between October 2003 and July 2005. In all cases, diagnosis was confirmed by identification of a typical mutation in the Notch 3 gene. Complete study design has been detailed elsewhere. Clinical and demographic data were collected, including age, sex, history of hypertension (defined as self-reported diagnosis of hypertension or antihypertensive drug intake), systolic blood pressure, diastolic blood pressure, diabetes (1997 World Health Organization criteria), history of hypercholesterolemia (self-reported diagnosis of hypercholesterolemia or lipid-lowering drugs intake), smoking habits, alcohol intake, and body mass index. Laboratory measurements (which included complete blood count, glucose, hemoglobin A1c, homocysteine, high-density lipoprotein, low-density lipoprotein, and total cholesterol levels) were performed in all patients. All subjects underwent detailed baseline neurological examination during the 2 hours before MRI examination, including a Mini-Mental State Examination and Mattis dementia rating scale. Degree of disability was assessed using the modified Rankin scale and Barthel Index. An independent ethics committee in both participating centers approved the study protocol.

**MRI**

MRI scans were obtained using a 1.5-T system (Vision; Siemens, Munich, Germany, or Signa; General Electric Medical Systems, Paris, France). Three-dimensional T1-weighted axial sequences (Munich: TR/TE 3300/15 ms, slice thickness 5.5 mm, interslice gap 1.5 mm, 128×256; Paris: TR/TE 8200/83 ms, slice thickness 5.5 mm, interslice gap 1.5 mm, 128×256, b value 1000 s/mm$^2$) were performed. Diffusion-weighted imaging scans were acquired in the X, Y, and Z directions and then averaged to make apparent diffusion coefficient (ADC) maps largely independent of the effects of anisotropic diffusion. Apparent diffusion coefficient values were then calculated to generate ADC maps as described elsewhere.

**Image Processing and Analysis**

**Lacunar Volume**

To assess the volume of each lacunar lesion, T1-weighted images were segmented in 4 tissue classes consisting of white matter, cortical and deep gray matter, cerebrospinal fluid (CSF) intensity lesions, and nonbrain partitions. After segmentation, the class of voxels corresponding to “CSF intensity lesions” was automatically isolated from the other tissue classes. Large-vessel infarctions were first excluded. Subsequently, 2 raters isolated lacunes from the other CSF voxels using appropriate 2- and 3-dimensional imaging tools. All hypointense lesions with both a signal identical to that of CSF on T1-weighted images and a diameter larger than 2 mm were selected for volumetric calculation. The volume of each segmented lesion and the number per patient were then recorded. In addition, the normalized total volume of lacunes in each patient was calculated as follows: ([total volume of lacunes/intracranial cavity volume]$^*$100). Good interrater reliability was previously reported for both the measurement of the total volume and estimation of the number of lacunes (intraclass correlation coefficient=0.830 and 0.824).

**White Matter Hyperintensity and Cerebral Microbleed Quantification**

The quantification of other lesions was made as previously described. Briefly, WMH were analyzed on all axial fluid-attenuated inversion recovery slices from the base of the cerebellum to the vertex. The total volume of WMH was normalized to the intracranial cavity in each patient (normalized volume=([volume of WMH/ volume ICC]$^*$100). The number of cerebral microbleeds (CMs), defined as rounded foci 5 mm or less in diameter hypointense on gradient echo sequences and distinct from vascular flow voids, leptomeningeal hemosiderosis, or nonhemorrhagic subcortical mineralization, was recorded separately.

**Mean Apparent Diffusion Coefficient and Brain Volume Assessment**

The estimation of mean cerebral diffusion and brain atrophy have been previously reported. The mean cerebral ADC was derived for each patient from histograms of ADC values obtained after exclusion of voxels containing CSF.

Cerebral atrophy was determined as the brain parenchymal fraction (BPF) corresponding to the ratio of brain tissue volume to total intracranial cavity volume (BPF=brain tissue volume/intracranial cavity volume). Automated determination of the volume of the intracranial cavity was done on proton density images from the base to the top of the skull. Global brain volumes were obtained from 3-dimensional T1 sequences using the Brainvisa software (CEA, Orsay, France; http://brainvisa.info).

**Statistical Methods**

In all analyses, ILV was the outcome of interest. Random-effect linear models were used to study the factors, both imaging markers (WMH, CM, mean ADC and BPF, total lacunar volume and number) and clinical characteristics (sex, age, blood pressure, cholesterol, diabetes) associated with ILV. Because one patient can have more than one lacune and therefore all lacunes are not independent from each other, this model allows to take into account intralacunar correlation. All imaging markers (explanatory variables) were studied both as continuous and categorical variables using quartiles of the distribution, except for the total number of lacunes per patients that was studied both as continuous and as 8 categories variables according to the following cutoffs: <5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 35, 35 to 70, and ≥ 70.

To characterize the distribution of lacunes volume per patient, we calculated the mean and median. We modeled how unitary lacunar volumes correlate with patients’ mean and median lacunar volumes. For that purpose, for a given lacune j of patient i, mean and median
were calculated based on all lacunes volumes belonging to patient i except lacune j.

All analyses were adjusted for sex and age and were performed using SAS (release 9.1; SAS Statistical Institute, Cary, NC).

Results
Among the 147 patients from the cohort, 129 patients had full sets of 3-dimensional T1, fluid-attenuated inversion recovery, proton density, T2*, and diffusion-weighted images of sufficient quality for postprocessing measurements. One hundred thirteen patients had one or more lacunar lesions. Their main demographic, clinical, and MIRI parameters are presented in the Table.

ILV distribution for 1568 segmented lacunes is shown in Figure 1. ILV varied from 4.28 mm³ to 1619 mm³ (median, 25.7) and was less than 500 mm³ for 90% of lacunes. For 15 lacunes, volume ranged between 1000 and 1619 mm³. Six ischemic lesions were of a volume larger than 1767 mm³ (volume of a sphere of 15 mm in diameter). These lesions were excluded from analysis.

The ILV was not found to be significantly associated with age, systolic or diastolic blood pressure, total cholesterol level, or the presence of diabetes. In addition, no significant association was observed between the ILV and the following parameters: WMH volume, number of CM, mean ADC (derived from whole histograms), and BPF.

ILV was strongly associated with the total volume of lacunes per patient (P = 0.003). No significant association was observed between the ILV and number of lacunes per patient.

Then, we investigated the relationship between ILV and the mean of ILV values obtained in each patient. ILV was significantly associated with the mean ILV per patient (F = 112.3, P < 0.0001; Figure 2). This relationship remained highly significant even after exclusion of the volume of the lesion of interest (corresponding to ILV) for the calculation of the mean ILV per patient (P = 0.0002, F = 6.54). A significant relationship was also observed between ILV and the median ILV per patient (F = 85.7, P < 0.0001). The normalization of each ILV value by the intraclass correlation coefficient did not modify these results.

Finally, we also analyzed the MRI data of patients with the largest lacunes (volume of at least one lacune larger than the 95th percentile of the distribution (> 306.14 mm³). Forty-six patients met this criterion. Median ILV of these patients was higher than the median ILV of the other 67 patients (71.0 mm³ versus 36.1 mm³, P = 0.03). It should be noted that small lacunes were also found in this subgroup of patients having at least one large lacune. The volume of the smallest lacune for each of these 46 patients varied between 4.28 and 146.1 mm³. We did not individualize patients with only large or small lesions, but observed patients with predominating small lacunes and others with predominating large lacunes.

Discussion
In this study of 1568 lesions identified among 113 patients with CADASIL, we observed that individual volume of lacunes varied between 4.28 mm³ and 1619 mm³. The analysis of the number of lesions according to their size showed that the distribution was skew to small ILV values with more than 90% of lacunes being <500 mm³. These findings confirm the results obtained in a previous pilot study in 10 patients and further emphasize that the vast majority of lacunes in CADASIL have a volume less than one third of the volume of a sphere of 15 mm in diameter, the upper limit usually chosen for the definition of these lesions on 2-dimensional imaging.

The results also showed a strong correlation between ILV and both mean and median volume of lacunes estimated at the patient’s level. These findings suggest that, at the patient level, the volume of a given lacune is related to the volume of the others. The results also indicate that the distribution of the volume of lacunes can considerably differ among patients because both median and mean lacunar volumes distribution vary greatly between patients. Interestingly, the ILV was found to be strongly associated with the total volume of lacunes. These results emphasize the importance of volumetric measurements in the exact estimation of the total burden of lacunar lesions.

In the present study, we did not observe any association between ILV and MRI markers of tissue lesions such as WMH volume and CM number or between ILV and markers of tissue loss such as mean ADC and BPF.
lacunes were observed in patients with extensive white matter lesions as well as in patients with only few hyperintensities. Moreover, ILV was not influenced by age. Finally, ILV was not found to be associated with the number of lacunes, which does not support the hypothesis that confluence of lesions occurring with the accumulation of lacunes leads to large cavities. Altogether, these results suggest that ILV in patients with CADASIL is not directly related to the progression of the vascular disease or to the atrophy process, but may be associated with other undetermined individual factors. The branching patterns of perforating arteries may be involved in the large variance of sizes of SVD infarcts. Other factors such as genetic variability may also account for these differences. The results of a recent study suggest the existence of a strong modifying influence of genetic factors distinct from the causative NOTCH3 mutation on the amount of ischemic brain lesions. Whether genetic factors can also influence the size of lacunar infarctions requires further investigation.

There are potential limitations in the interpretation of our results. First, the selection of lacunes was based on 3-dimensional semiautomatic segmentation on T1-WI without pathological verification. Therefore, we cannot exclude that some of segmented objects, especially the smallest lacunes, were in fact Virchow-Robin spaces. However, to reduce this potential misclassification bias, lesions with a diameter less than 2 mm were excluded from the study. In addition, variables were analyzed in this study as continuous and categorical using quartiles of the distribution and the results were found to be significant over the whole spectrum of ILV. On the other hand, 6 segmented lesions observed in 4 patients with CADASIL had their volume larger than the usual volumetric limits of lacunar infarctions. Because we
cannot exclude another origin for these lesions, they were not considered in the statistical analysis. Second, because our study was cross-sectional, we cannot definitively exclude an effect of disease progression on ILV. Prospective studies are needed to establish whether modifications of ILV can occur in vivo with the progression of SVD. Finally, we possibly underestimated the exact size of ischemic lesions because we chose to analyze only areas with MRI signal identical to that of CSF. Although lacunar infarctions are most often observed as focal areas of complete tissue necrosis with cavitation (Type 1a lacune), they can also present with incomplete tissue loss and limited cavitation (Type 1b or incomplete small infarct).  

In summary, in this large cohort of patients with CADASIL, we found a strong relationship between the ILV and both the mean and median of lacunar volume distribution at the patient’s level. Our results suggest an individual predisposition for having mainly small lacunes in some subjects and larger lesions in others. The origin of these differences remains unknown and does not seem to be related to the severity of other ischemic lesions.

Disclosures

None.

References
