Cerebral microinfarcts (CMIs), small ischemic lesions seen microscopically during pathological examination of cerebral tissue, have been implicated as important contributors to vascular cognitive impairment. Estimating an individual’s total CMI burden would be an important step toward defining the impact of these lesions. This effort is hampered, however, by our inability to reliably detect CMI or CMI-related changes with conventional neuroimaging because of their microscopic size. Postmortem analysis of CMI is also limited by the practical impossibility of microscopically sectioning through the whole brain. To address this limitation, we recently reported a method for estimating total CMI burden from brain tissue sampled at autopsy. This method is available at http://stroke.ahajournals.org.

An alternative approach applicable to living subjects is to estimate CMI burden from the presence of hyperintense lesions on magnetic resonance imaging (MRI) diffusion-weighted imaging (DWI). These DWI hyperintensities have been suggested to represent relatively large acute/subacute CMI and remain detectable for ≈1 to 2 weeks. These DWI hyperintense lesions occur with increased incidence in patients with primary intracerebral hemorrhage (ICH), suggesting that severe small vessel disease (SVD), causing ICH, might also give rise to silent CMI.

We now describe a simple mathematical method for estimating total number of CMI based on the presence of

### Background and Purpose

Cerebral microinfarcts (CMIs) are important contributors to vascular cognitive impairment. Magnetic resonance imaging diffusion-weighted imaging (DWI) hyperintensities have been suggested to represent acute CMI. We aim to describe a mathematical method for estimating total number of CMI based on the presence of incidental DWI lesions.

### Methods

We reviewed magnetic resonance imaging scans of subjects with cognitive decline, cognitively normal subjects and previously reported subjects with past intracerebral hemorrhage (ICH). Based on temporal and spatial characteristics of DWI lesions, we estimated the annual rate of CMI needed to explain the observed rate of DWI lesion detection in each group. To confirm our estimates, we performed extensive sampling for CMI in the brain of a deceased subject with past lobar ICH who found to have a DWI lesion during life.

### Results

Clinically silent DWI lesions were present in 13 of 343 (3.8%) cognitively impaired and 10 of 199 (5%) cognitively intact normal non-ICH patients, both lower than the incidence in the past ICH patients (23 of 178; 12.9%; P < 0.0006). The predicted annual incidence of CMI ranges from 16 to 1566 for non-ICH and 50 to 5041 for ICH individuals. Histological sampling revealed a total of 60 lesions in 32 sections. Based on previously reported methods, this density of CMI yields an estimated total brain burden maximum likelihood estimate of 9321 CMIs (95% confidence interval, 7255–11 990).

### Conclusions

Detecting even a single DWI lesion suggests an annual incidence of hundreds of new CMI. The cumulative effects of these lesions may directly contribute to small-vessel–related vascular cognitive impairment. DOI: 10.1161/STROKEAHA.115.009208.
incidental DWI hyperintense lesions, using data on the incidence of these lesions in individuals with ICH, cognitive decline, or cognitively intact elderly controls. To test the plausibility of our estimates, we measured the number of CMI in brain tissue sections from a subject with a DWI lesion during life.

Methods

Study Participants

To obtain data on the incidence of DWI lesions in different patient groups, we reviewed 398 subjects with documented cognitive decline and 204 similar aged cognitively normal subjects. The cognitive decline group was enrolled in an ongoing prospective longitudinal study at the Massachusetts Alzheimer’s Disease Research Center between 2007 and 2010 and underwent at least 1 MRI scan. For the present study, we excluded subjects with only subjective cognitive complaints and mean global Clinical Dementia Rating (CDR). Scale of 0 and subjects with a history of ICH. Of the 398 Massachusetts Alzheimer’s Disease Research Center patients, we excluded 31 lacking adequate quality DWI images, 13 with other incidental brain pathologies, 9 with global mean CDR 0 and 2 with potential stroke symptoms at time of scanning, leaving 343 subjects for analysis, including 173 (50%) diagnosed with mild cognitive impairment, 103 (30%) with Alzheimer disease, 20 (6%) with mixed or vascular dementia, and 47 (14%) with cognitive impairment because of other pathogenesis including frontotemporal dementia, Parkinson’s disease, and diffuse Lewy body dementia. The cognitively normal subjects are community-dwelling participants in the ongoing longitudinal Harvard Aging Brain Study previously described. As part of the selection process for this study, participants were excluded for previously diagnosed neurological or psychiatric conditions including ICH. Of the 204 cognitively normal subjects, we excluded 3 lacking DWI sequences and 2 with other incidental brain lesions, leaving 199 for analysis. A third group of subjects comprised patients with past ICH, described previously in detail. Of 392 subjects with history of ICH, we analyzed 178 subjects with DWI images. For patients who underwent >1 scan, we selected the chronologically first scan performed at least 2 weeks post ICH.

Demographic and clinical data including age, sex, and presence of vascular risk factors at the time point closest to MRI were obtained by chart review for the cognitive decline group and from a study questionnaire completed by control subjects. Data on CDR and type of dementia were determined by the Massachusetts Alzheimer’s Disease Research Center–treating physician. Control subjects underwent neuropsychological testing as described.

Numbers of missing covariates are indicated in Table 1. All aspects of the study were approved by the hospital institutional review board and all participants provided informed consent.

MRI Acquisition and Analysis

Cognitive decline and past ICH subjects underwent brain MRI on a 1.5 Tesla (T) Sigma scanner (GE Medical Systems, Milwaukee, WI) using DWI, fluid attenuation inversion recovery (FLAIR), and gradient-recalled echo (GRE) as previously described. A subset of cognitive decline subjects (n=89) had MRI performed on a 3T Trio scanner (Siemens, Munich, Germany), using DWI (repetition time/echo time, 6230/84 ms; slice thickness, 3.0 mm; interslice gap, 1 mm; 128 x 128; b value, 700 s/mm²), FLAIR, and GRE images as described. Cognitively normal subjects underwent MRI on the same 3T scanner, using DWI (repetition time/echo time, 8040/84 ms; slice thickness, 5.5 mm; interslice gap, 1 mm; 128 x 128; b value, 700 s/mm²), FLAIR, and GRE sequences as described.

All images were reviewed for the presence, number, and distribution of hyperintensities on DWI as well as cerebral microbleeds on GRE and severe white matter hyperintensities (WMH) as previously reported. We previously reported high inter-rater reliability for DWI lesion detection (κ=1). A composite image of the spatial distribution of DWI lesions was created as described.

Estimating CMI Incidence From DWI Lesions

We developed mathematical models to estimate the underlying incidence of all CMI from detection of the subset of new lesions visible to DWI. For the purposes of our model, we assumed that detectability of CMI on DWI depends on CMI size, spatial resolution of the scan, location of the CMI relative to MRI slices, and timing of the scan relative to CMI genesis. With these in mind, we further assumed (1) that incidental small DWI lesions correspond to acute CMI, (2) only lesions that occur within 10 days preceding MRI are detectable, yielding a probability that a new lesion will occur within the detectability time window on a randomly timed MRI scan of \( P = \frac{\text{annual rate of lesions} \times \text{probability of detection}}{\text{probability of lesion occurrence}} \), (3) the annual rate of lesions (n) is an independent variable, and (4) CMI lesions, modeled as small spheres, can be detected only if their cross-sectional profile that falls within an imaged MRI slice is large enough to be detected as a DWI hyperintensity. We defined the variable \( \gamma \), the proportion of CMI with large enough imaged profiles to be detectable on MRI, to reflect the proportion of CMI that are large enough for MRI detection (\( \kappa \)) multiplied by the probability that a detectably large CMI actually falls into an imaged MRI slice (\( \delta \)). Based on slice thickness parameters in the analyzed DWI scans, we calculated that \( \delta \) would range between 0.8 and 1 under all conditions and therefore made the simplifying assumption that \( \gamma \approx 1 \), that is, essentially all CMI large enough to be detectable would fall within an imaged slice.

Previous pathological studies reported a mean CMI diameter of \( \approx 0.2 \) mm, whereas the in-plane resolution of DWI-MRI is at least

<table>
<thead>
<tr>
<th>Table 1. Subjects With Cognitive Impairment, Controls, and Past ICH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Impairment (n=343)</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Sex (female)</td>
</tr>
<tr>
<td>DWI lesion present</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
</tr>
<tr>
<td>CMB, median (interquartile range)</td>
</tr>
<tr>
<td>Severe WMH</td>
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</tbody>
</table>

Significance, after Bonferroni correction, was set at \( P < 0.016 \). Values are mean±SD or n (%). CMB indicates cerebral microbleeds; DWI, diffusion-weighted imaging; ICH, intracerebral hemorrhage; and WMH, white matter hyperintensity. Missing variables: CMB, 49; WMH, 15; hyperlipidemia, 5; Diabetes mellitus, 3; and hypertension, 1.*"
an order of magnitude larger. Even allowing for some enlargement or blooming of the DWI lesion relative to the underlying size of the lesion,\textsuperscript{19} a CMI would likely need to be on the order of 1.0 mm diameter to be detectable. Based on data from the Religious Orders Study,\textsuperscript{2,17} the percentage of CMIs expected to have diameters $\geq 1.0$ mm is $\approx 0.5\%$ (Figure 1 in the online-only Data Supplement). We therefore considered values for $\gamma$ down to a lower limit of 0.1\% and up to an upper limit of 10\% to allow for analysis of conservative assumptions (ie, that would yield lower estimates for CMI incidence). See Methods in the online-only Data Supplement.

A similar approach was applied to determine the maximum likelihood estimate for the underlying rate of incident CMI expected to generate $\geq 1$ DWI lesions on a single scan as well as the impact of negative scans on annual lesion rate (see Methods in the online-only Data Supplement).

Pathological Examination for CMI

To test the plausibility of our estimations, we performed extensive sampling for CMI of the brain of a 56-year-old man enrolled to a prospective cohort study of cerebral amyloid angiopathy.\textsuperscript{20} Seven years before his death he had spontaneous left parietal ICH and gradually developed cognitive decline since. Imaging revealed 10 lobar cerebral microbleeds. Two years before death, the subject was found to have 1 incidental DWI lesion on a research scan. No lesions were detected on a DWI scans performed 10 months before and 14 months after the DWI-positive scan. After autopsy, the left hemisphere was systematically sampled in 32 sections, which were examined for the presence and number of CMI (see Methods in the online-only Data Supplement).

Statistical Analysis

Comparisons between subjects with ICH, cognitive decline and controls were performed by 1-way ANOVA and t test, respectively (for age), Wilcoxon rank-sum (for WMH, CDR, and cerebral microbleed), and $\chi^2$ or Fisher exact tests (for categorical variables). Bonferroni-corrected threshold for the 3 between-group comparisons was set at $P=0.016$. Multivariable models were analyzed by logistic regression. The maximum likelihood estimate analysis was done with software written by the authors using Matlab (Natick, MA).

Results

Lesion Incidence and Distribution

The cognitively impaired and cognitively normal non-ICH patients were of similar age to each other (Table 1) and slightly older than the past ICH patients.\textsuperscript{5} Clinically silent DWI lesions were present in 13 of 343 (3.8\%) cognitively impaired and 10 of 199 (5\%) cognitively normal non-ICH patients, both lower than the incidence in the past ICH patients (23 of 178; 12.9\%; $P<0.0006$ for both comparisons). Four (1.2\%) cognitively impaired and 3 (1.5\%) cognitively intact patients had a corresponding low absolute diffusion coefficient signal suggesting an acute time course, whereas the remainder were isointense on absolute diffusion coefficient consistent with subacute infarcts. The differences between lesion incidence in the ICH and 2 non-ICH groups remained independent in analyses controlling for age, sex, and hypertension ($P<0.0013$ for both comparisons). The incidence of DWI lesions did not differ between cognitively impaired subjects and normal controls ($P=0.49$) or between subjects with mild (CDR=0.5) or more severe (CDR $\geq 1$) cognitive impairment ($P=0.6$). No association was found between the presence of DWI lesions and severe WMH ($P=0.32$).

Five of the non-ICH subjects had multiple (2–3) lesions, yielding a total of 30 lesions, primarily in the cortical gray matter or gray/white border (14 lesions; 47\%) or subcortical white matter (6 lesions; 20\%). Of the remainder, 7 (23\%) were cerebellar, 2 (6.7\%) brain stem, and 1 deep hemispheric (3.3\%; Figure 1). The 20 lobar lesions were slightly over-represented in the occipital and underrepresented in the temporal lobes relative to the expected random distribution by lobar volume ($P=0.046$; Table 2) and did not differ between the cognitive impairment group and controls (Figure 1; $P=0.9$).

Estimation Model

Our mathematical model estimates the likely annual rates of new CMI that would produce the observed fraction of subjects with at least 1 incident DWI lesion. As shown in the curves in Figure 2, the estimate depends on the parameter $\gamma$, representing the proportion of all CMI that are detectable by DWI. The 3 curves in Figure 2 correspond to 3 plausible values for this parameter, ranging from 0.1\% to 10\%. To generate the observed proportions of 4.2\% (for elderly subjects without ICH) or 12.9\% (for subjects with past ICH), the predicted

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Distribution of diffusion-weighted imaging (DWI) lesions. The axial (A) and sagittal (B) images display the composite cerebral and cerebellar locations of the DWI lesions in the cognitively impaired (red) and normal control subjects (green). Each spot represents the center of the lesion.}
\end{figure}
annual incidence of CMI ranges from 16 to 1566 for non-ICH and 50 to 5041 for ICH individuals. An estimate for total CMI burden can then be calculated by multiplying these annual rates by the years an individual can be expected to live at a given state. At \( \gamma=1\% \), for example, a non-ICH patient would be predicted to accumulate 1570, 3140, and 4710 CMIs for 1, 2, or 3 decades, respectively.

The same mathematical approach can be used to calculate maximum likelihood estimate for annual CMI incidence based on the presence and number of observed DWI lesions (Figure 3). At \( \gamma=1\% \), for example, the model predicts an annual incidence of 3649 (95% confidence interval, 193–16 422) new CMI per year for individuals with 1 DWI lesion on a single scan and 7299 (95% confidence interval, 1399–21 270) for the presence of 2 DWI lesions. For \( \gamma<1\% \) (ie, <1% of incident CMI detectable as DWI lesions), the predicted range for new CMI per year would be correspondingly greater.

**Neuropathologic Validation**

A subject with a single DWI lesion on 1 of 3 scans during life had autopsy with extensive tissue sampling 2 years after the DWI-positive scan (see Methods section of this article). Extensive histological sampling revealed a total of 60 lesions (Figure 4) in 32 sections, corresponding to a mean lesion per slide density of 1.9±1.3 with approximately normal distribution across the examined slides (Figure II in the online-only Data Supplement). Based on previously reported methods,\(^2\) this density of CMI in the sampled tissue yields an estimated total brain burden maximum likelihood estimate of 9321 CMIs (95% confidence interval, 7255–11 990). For comparison, the 95% confidence interval range of annual predicted CMI incidences at \( \gamma=1\% \) for a single DWI lesion, adjusted for the 2 additional negative DWI scans during life (Table I in the online-only Data Supplement), is 1051 to 2529. The estimate for total CMI burden obtained from pathological sampling is thus compatible with the estimate for annual incidence obtained from neuroimaging for a 3.7- to 8.9-year period of lesion accumulation, a timeframe supported by a recent survival analysis of patients diagnosed with advanced cerebral amyloid angiopathy.\(^2\)

**Discussion**

In this study, we found that subclinical DWI lesions occur at appreciable frequency in cognitively normal or impaired elderly individuals, although at lower frequency (and somewhat different location) relative to subjects with past ICH. A second notable finding from the current analysis is that because of the limited spatial and temporal sensitivity of diffusion-weighted MRI, the chance detection of even 1 or 2 DWI lesions suggests an annual incidence of hundreds of new CMI. These findings add data from living patients to the growing evidence for the sizable burden posed by CMI in the aging brain.

Measuring the full burden of CMI has been challenging. High field strength (7T) structural imaging\(^2\) is a promising approach, but has to date been validated (via ex vivo imaging–pathological correlation) to detect only the largest CMI in the range of 1 to 2 mm diameter. The currently described approach of DWI suffers similar limitations of spatial resolution as well as limited temporal resolution and further has yet to be validated as detecting pathological CMI. It is nonetheless notable that our estimate of total CMI burden expected to accumulate

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**Table 2.** DWI Lesions Distribution by Cerebral Lobe in Non–Intracerebral Hemorrhage Subjects

<table>
<thead>
<tr>
<th>Observed Distribution of Lobar DWI Lesions, n (%)</th>
<th>Lobar Volume, %(^2)</th>
<th>Ratio Observed/Expected</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal 8 (40)</td>
<td>40.6</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>Parietal 6 (30)</td>
<td>22.6</td>
<td>1.3</td>
<td>...</td>
</tr>
<tr>
<td>Temporal 0</td>
<td>22.8</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>Occipital 6 (30)</td>
<td>13.9</td>
<td>2.2</td>
<td>0.046</td>
</tr>
</tbody>
</table>

DWI indicates diffusion-weighted imaging.
for 1 decade in non-ICH patients (1570 at $\gamma=1\%$) is in the same range as the estimates arrived at independently based on finding 1 or 2 CMI in routine neuropathologic specimens (552–1104). The subset of individuals with DWI lesions detected on an MRI scan are predicted to have even greater CMI burden, with annual incidences in the range of 3649 to 7299 for those with 1 or 2 lesions (Figure 3). This high rate of CMI accumulation is supported by the single extensively sampled postmortem brain from a patient who was DWI-positive during life, estimated to have >9000 total microinfarcts. Another support for our estimation was recently shown by Conklin et al. In that study, 5 patients with WMH underwent brain MRI scans for 16 consecutive weeks. Nine new DWI lesions were detected during the study period in 3 of the 5 subjects.

The prevalence of DWI lesions in our study is greater than previously reported. In a study of 16206 consecutive patients who underwent MRI, the reported prevalence was 0.37%. This study, however, looked at both young and elderly patients and therefore substantially differs from our cohort. Another study of 649 subjects recruited from a memory cohort reported a prevalence of 0.9%. Both studies focused on acute DWI lesions only and required DWI lesions to be dark on absolute

Figure 3. Estimated cerebral microinfarct (CMI) rate as function of number of diffusion-weighted imaging (DWI) lesions. Maximum likelihood estimates ([MLE] with 95% confidence intervals [CIs]) are shown for 0, 1, 2, and 3 DWI lesions detected on a single scan ($\gamma=1\%$).

Figure 4. Cerebral microinfarcts (arrows) in diffusion-weighted imaging (DWI)–positive cerebral amyloid angiopathy patient. Tissue sections from a 56-year-old man with a DWI lesion detected during life show a linear cortical microinfarct and 2 distinct white matter infarcts (A), a single remote cortical infarct (B), a subacute white matter infarct with presence of myelin-laden macrophages (C), and a remote white matter microinfarct (D). A, C, and D, Luxol fast blue-hematoxylin–eosin; B, hematoxylin–eosin; scale bar is 500 $\mu$m in A, 200 $\mu$m in B to D.
diffusion coefficient, considerably shortening the time window of detectability.\textsuperscript{3,4}

The exact mechanism and underlying pathophysiology leading to small silent ischemic lesions are not yet well understood. These lesions may be caused by SVD (arteriosclerosis or cerebral amyloid angiopathy), microemboli, cerebral hypoperfusion, endothelial dysfunction, vasostriction, or chronic inflammation. As our analysis did not include subjects with acute ICH, immediate post-ICH factors such as aggressive blood pressure reduction, failure of autoregulation, or increased intracranial pressure\textsuperscript{5,6,27} are less likely to play a role. The greater prevalence of lesions in patients with past (nonacute, >14 days) ICH thus suggests an important role for the SVDs associated with ICH.

Strengths of our study include the number of subjects analyzed. All scans across the groups (including ICH subjects from our previous study\textsuperscript{8}) were reviewed by the same 2 assessors blinded to clinical data. Our pathological method for sampling to detect CMI was selected as a rough histological approximation of neuroimaging, in that the samples were drawn from approximately evenly space slabs cut along the anterior–posterior axis of the hemisphere and sampled without regard for evident lesions or anatomic targets. In this manner, we have attempted to perform an unbiased sampling. In addition, the degree of sampling exceeded that routinely performed in neuropathologic assessment.

Major limitations included our study’s retrospective, cross-sectional design and the absence of MR-pathological correlation to confirm that DWI-positive lesions indeed represent CMI. However, we recently show\textsuperscript{28} that these lesions nonetheless caused substantial changes on diffusion tensor imaging, lasting after their disappearance, consistent with the interpretation that they represent CMI. A limitation of the retrospective neuroimaging data was the mix of 1.5T and 3T magnetic field strengths among the study subjects. DWI lesion detection did not differ between the cognitive decline subjects scanned at 1.5T versus 3T ($P=0.2$) or between individuals within a patient group scanned with different interslice gaps ($P=0.74$), however, consistent with a previous analysis showing no gain in DWI sensitivity at the higher field strength.\textsuperscript{29} Another area of limitation is the large number of simplifying assumptions required for this type of mathematical modeling of biological phenomena. We assumed, for example, that CMI lesions occur independently over time. A tendency of CMI to cluster in time, such as during the timeframe immediately after ICH,\textsuperscript{6,8,11} may lead to overestimation, if the scan is taken during a time of increased DWI occurrence, or underestimation if during a relatively quiescent period. It is nonetheless reassuring that scans of the controls ($n=199$) were performed strictly for research purposes and were not triggered by any clinical event, reducing the possibility of scanning in proximity to such a precipitating event. Another limitation is the unknown fraction ($\alpha$) of CMI large enough for detection by MRI. Based on the small reported size distribution of pathologically detected CMI (average diameter $\approx 0.2$ mm),\textsuperscript{2,8,30} the 1% to 10% estimates seem unlikely to be too low and may indeed be conservatively high (leading to underestimation rather than overestimation of total CMI burden). As the real value of $\gamma$ is unknown, we tested a wide range of values and all of them produced high estimates for total burden. The large number of CMI counted in our pathological sample also argues for the validity (and conservative parameter estimates) of the current model.

Finally, we note that the similar frequencies of DWI-positive scans among cognitively impaired and intact elderly subjects does not support the suspected role for these lesions in promoting cognitive impairment.\textsuperscript{30,31} A possible explanation for the lack of correlation with cognitive impairment is the wide range of total CMI burdens compatible with the finding of a single DWI lesion, evidenced by the 95% confidence intervals surrounding the above maximum-likelihood estimates. We also note that the cognitively impaired group included few subjects ($n=20$) diagnosed with vascular dementia or mixed dementia, potentially the likeliest groups to demonstrate aggressive SVD as contributor to their impairments.

The accumulating evidence for large burdens of CMI in the aging brain highlights the importance of determining these small lesions’ impact on neurological function. Silent DWI lesions have been linked to cerebrovascular events or vascular death\textsuperscript{9} or poor long-term functional outcome\textsuperscript{10,26} after acute ICH and with increased risk of recurrent ischemic stroke, transient ischemic attack, and vascular death after acute ischemic stroke.\textsuperscript{32} Future studies will determine whether DWI lesions might be incorporated (along with lacunes, WMH, perivascular spaces, and cerebral microbleed) to the proposed SVD score\textsuperscript{13} or contribute independent information on SVD severity for longitudinal studies or interventional trials.

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Drs Greenberg, Auriel, Westover, Bianchi, and Gurrol contributed to study design; Dr Auriel, Dr Gurrol, A. Vaskhechiv, Dr Reijmer, Dr Martinez-Ramirez, Dr Frosch, Dr Sperling, Dr Johnson, A.P. Younger, Dr Hedden, and P. Fodiids to data acquisition; Drs Auriel, Westover, Bianchi, and Greenberg to data analysis; Dr Greenberg, K. Schwab, and A. Vaskhechiv to study management; Drs Auriel, Greenberg, Westover, and Bianchi to article preparation; and all authors contributed to article review.

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Disclosures

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References


Estimating Total Cerebral Microinfarct Burden From Diffusion-Weighted Imaging
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Data Supplement (unedited) at:
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SUPPLEMENTAL MATERIAL

Estimating total cerebral microinfarct burden from diffusion-weighted imaging

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Supplementary Methods

Neuropathologic examination

Following autopsy of this 56 year old man, the left hemisphere was fixed in buffered formalin and cut in the coronal plane into 16 0.5-to-1cm thick slabs as described ¹. Two standard ("1x3" glass slide) paraffin blocks were taken from each slab, one from the lateral aspect and the other from the medial surface. The samples of the lateral aspect of the hemisphere were aligned along the anterior-posterior axis to follow the contour of the middle frontal gyrus with continuity along the lateral aspect of the hemisphere to the occipital pole. Those on the medial aspect were taken at the same level along the superior-inferior axis but along the medial surface of the hemisphere. Because the spacing of slabs was partially driven by anatomic landmarks (optic chiasm, mammillary bodies, etc), tissue sampling from the slabs should be considered as semi-unbiased.

All blocks were routinely processed and stained with LH&E or H&E. Slides were reviewed by three observers including one experienced diagnostic neuropathologist (EA, YDR, MPF), and CMI were counted on each slide. Diagnostic features for a CMI were evidence of loss of normal cells (neurons, oligodendrocytes) and reactive gliosis; more acute lesions often contained macrophages with debris. Lesions with substantial hemosiderin were not included, as these were interpreted as CMB. A histogram of the number of CMIs counted per tissue section is shown in Figure II.

Estimation Model

Our binomial model implies that the probability of any single lesion occurring within the detectability temporal window, being large enough to be detected and occurring within the slices where is $pγ$. The probability that at least one of the $n$ lesions that occur each year is detected is equal to the complement of the probability that none of the $n$ lesions are detected. This latter probability is the probability that any given lesion goes undetected, $(1-pγ)$, multiplied by itself $n$ times, $(1-pγ)^n$ (as CMIs are independent events). Therefore, the complement is
$$f(n; p, \gamma) = 1 - (1-p\gamma)^n$$

We also analyzed the converse question: If we see a single DWI lesion, what is the most likely underlying rate or range of rates at which lesions may be accruing? We estimate this by maximum likelihood estimate (MLE): we calculate the probability of seeing \( \kappa \) DWI lesions on a single random scan as a function of the underlying annual rate \( n \). In our model the probability of detecting \( \kappa \) lesions on an MRI when the underlying rate is \( n \) (with \( p\gamma \) as the probability of detecting any one of these independent events) is:

$$h(\kappa|n, \rho, \gamma) = (\kappa) (\rho\gamma)^\kappa (1-\rho\gamma)^{n-\kappa}$$

To assess the additional effect of negative DWI scans on the MLE, and 95% confidence intervals, we generated \( 10^6 \) runs of Monte Carlo simulation over a very wide range of underlying CMI rate \( r \) (between 0 and 3650 lesions per year). For each run, new lesion occurrence over 10 day periods was simulated following a Poisson process (equal probability of occurrence at any given instant, and average rate equal to \( r \)). The MLE (or 95% CI) for having the observed combination of 1 DWI-positive and 2 DWI-negative scans (Table I) were calculated from the underlying lesion rate that most often produced this combination of scan results (or that contained 95% of the trials producing this combination of results) over the \( 10^6 \) simulated runs.
Table I. Effect of negative DWI scans on annual lesion rate.

<table>
<thead>
<tr>
<th>DWI-positive MRI scans/Total MRI scans</th>
<th>Underlying annual rate of CMI (95% CI)*</th>
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</thead>
<tbody>
<tr>
<td>0/3</td>
<td>0-1050</td>
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* Estimates performed at $\gamma=1\%$
Supplemental Figure Legends

**Figure I** Size distribution of CMI diameters. The plot shows the kernel estimate of the distribution of CMI diameters generated from measurements performed in the Religious Orders Study. The actual measured diameters are shown as red ‘+’ signs below the estimated distribution.

**Figure II** Numbers of CMI per pathological slide from the brain of the 56 year old man with an incidental DWI lesion during life.
Figure I
Figure II


Estimating Total Cerebral Microinfarct Burden From Diffusion-Weighted Imaging

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Abstract

Background and Purpose: Cerebral microinfarction (CMI) is a significant determinant of disability in patients with ischemic stroke. Previous studies have used conventional MRI to estimate the number of microinfarcts. However, conventional MRI does not have the sensitivity to detect small lesions that may not be visible on T2-weighted or FLAIR images. Diffusion-weighted imaging (DWI) is a more sensitive method for detecting small lesions that may represent the earliest stages of infarction.

Methods: We used DWI to identify microinfarcts in 178 patients with acute ischemic stroke. The number of DWI lesions was correlated with the number of CMI lesions identified on conventional MRI. A novel method was developed to estimate the total number of microinfarcts based on the DWI data.

Results: The number of microinfarcts identified on DWI was strongly correlated with the number of CMI lesions identified on conventional MRI. The novel method was able to estimate the total number of microinfarcts with high accuracy.

Conclusion: Diffusion-weighted imaging is a promising tool for detecting cerebral microinfarcts, and the novel method developed in this study can be used to estimate the total number of microinfarcts in patients with ischemic stroke.

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