Age-Dependent Susceptibility to Infarct Growth in Women

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Background and Purpose—It is not known if there is a relationship between gender and tissue outcome in human ischemic stroke. We sought to identify whether the proportion of initially ischemic to eventually infarcted tissue was different between men and women with ischemic stroke.

Methods—We studied 141 consecutive patients with acute ischemic stroke who had a baseline MRI obtained within 12 hours of symptom onset, a follow-up imaging on Day 4 or later, and diffusion-weighted imaging/mean transmit time mismatch on initial MRI. Lesion growth was calculated as percentage of mismatch tissue that underwent infarction on follow-up (percentage mismatch lost). Multivariable analyses explored the effect of gender and other predictors of tissue outcome on percentage mismatch lost.

Results—There was no difference in median percentage mismatch lost between men (19%) and women (11%; P=0.720). There was, however, an interaction between gender and age; median percentage mismatch lost was 7% (0% to 12%) in women and 18% (1% to 35%) in men younger than the population median (71 years, P=0.061). The percentage mismatch lost was not different between men and women ≥71 years old (25% in both groups). The linear regression model revealed gender (P=0.027) and the interaction between age and gender (P=0.023) as independent predictors of percentage mismatch lost.

Conclusions—There is an age-by-gender interaction in tissue outcome after ischemic stroke; brain infarcts in women <70 years grow approximately 50% less than infarcts in their male counterparts. These findings extend the well-known concept that there is a differential age-by-gender effect on stroke incidence, mortality, and functional outcome to the tissue level. (Stroke. 2011;42:00-00.)

Key Words: cerebral infarct · diffusion-weighted imaging · gender · magnetic resonance imaging · outcome · perfusion-weighted imaging · women & minorities

Brain imaging currently plays an essential role in the diagnosis of stroke both in distinguishing between hemorrhage and ischemia and in determining the extent and localization of the lesion. Recent advances in imaging have provided the ability to assist not only in the diagnosis, but also to estimate the likelihood of irreversible injury within the ischemic territory.1 Diffusion-weighted MRI (DWI) depicts tissue with evidence of ischemic injury. Perfusion-weighted MRI (PWI) reveals regions with impaired tissue perfusion. The mismatch tissue that occurs between abnormal tissue visualized on acute DWI and acute PWI has been postulated to represent a tissue at risk.1 The outcome of the mismatch tissue is markedly variable in humans, ranging from no infarction, even without intervention (thrombolysis), to loss of all tissue at risk.2 This is consistent with the notion that not all brains can handle an ischemic insult of similar degree and duration in the same manner.3,4 Identification of determinants of tissue outcome in ischemic stroke is a step toward the understanding of evolution of stroke in humans and hence developing new potential therapeutic targets. Variables such as age,5 the severity and volume of leukoaraiosis,6 genetic susceptibility,7 time of imaging relative to symptom onset,8 and factors that alter the susceptibility to ischemic injury (arterial blood pressure, blood glucose, fever, etc) might explain some of the variance in tissue outcome.

Published data in animals suggest that gender is also important in determining the amount of ischemic tissue turning into infarction. Young female animals are more resistant to cerebral ischemia and have smaller lesion volumes after experimental ischemic brain injury.9,10 The favorable response to ischemia in female animals appears to diminish with age,11,12 suggesting a possible role for sex hormones in the protection against ischemic injury.13 The purpose of this study was to find out whether gender is a predictor of tissue outcome in human stroke. More specifically, we sought to understand whether the proportion of
initially ischemic but eventually infarcted tissue was different between men and women with ischemic stroke.

**Methods**

**Study Population**

We analyzed data that was prospectively collected as part of a National Institutes of Health-funded study evaluating the use of DWI and PWI in predicting tissue outcome in acute ischemic stroke (MRI Diffusion/Perfusion Mismatch in Human Acute Stroke). This study included consecutive patients with DWI and PWI performed within 12 hours of symptom onset who underwent a second imaging study (MRI or CT) on Day 4 or later to assess the final infarct volume during an 8-year period between 2000 and 2008. Both CT and MRI were used for follow-up assessment because they both conspicuously define infarct limits after 24 hours of stroke onset. Because of suboptimal reliability of percentage mismatch lost (PML) determination at small mismatch volumes, we excluded patients with small DWI/PWI mismatch defined as “mean transit time (MTT) volume/DWI volume <1.2” and “MTT volume/DWI volume <10 mL.”

We also excluded those in whom assessment of final infarct was not possible due to low-quality follow-up images, hemianciectomy, extensive hemorrhagic conversion, or massive brain edema and who received experimental or intra-arterial thrombolytic treatment. The study protocol was approved by the local Institutional Review Board.

**Data Collection**

Demographic (age, gender) and clinical data (history of hypertension, diabetes mellitus, coronary artery disease, congestive heart failure, atrial fibrillation, admission blood glucose, admission mean arterial blood pressure, intravenous tissue plasminogen activator treatment, time from symptom onset to MRI, admission stroke severity, etiologic stroke subtype) were collected. Time of stroke onset was defined as the last time the patient was seen normal. Admission stroke severity was assessed by the National Institutes of Health Stroke Scale score. Etiologic stroke subtype was determined using the Causative Classification of Stroke system.

**Image Acquisition and Analysis**

All acute and follow-up MR images were performed by 1.5-T (GE Medical Systems, Milwaukee, WI) or Siemens Sonata (Siemens Medical Solutions, Erlangen, Germany) scanners except for 10 patients who had their follow-up images obtained by 3-T GE Signa or Siemens Trio scanners. Image acquisition and processing protocols were described in detail previously.

Briefly, DWI was obtained using echoplanar imaging. Average DWI maps as well as apparent diffusion coefficient maps were computed from images corrected for motion and eddy-current distortions. Perfusion-weighted images were acquired using dynamic susceptibility contrast echoplanar imaging. MTT and cerebral blood flow maps were calculated as described previously.

The CT studies were performed by using a helical scanner (High-Speed Advantage; GE Medical Systems).

Ischemic lesions on admission DWI and MTT maps and on fluid-attenuated inversion recovery or CT images were manually outlined and lesion volumes were calculated using MRICro software (University of Nottingham). All lesion volumes were corrected for differences in overall brain size using midsagittal cross-sectional intracranial area as a surrogate measure of the intracranial volume.

All MRI measurements were performed by a neuroradiologist experienced in image analysis and processing (E.M.A.) blinded to the clinical data. Methods used for calculation of infarct volume as well as intracranial area have been previously reported to have high inter-rater reliability. The proportion of ischemic tissue undergoing infarction within the mismatch region was determined by PML and was calculated according to the following formula:

\[
PML = \frac{\text{follow-up lesion volume} - \text{DWI volume}}{\text{MTT volume} - \text{DWI volume}} \times 100.
\]

**Statistical Analysis**

Statistical analyses explored the relationship between gender and tissue outcome (PML). Bivariate relationships between clinical and imaging variables and tissue outcome were explored using Mann-Whitney U and Kruskal-Wallis tests for categorical variables and Spearman correlation for continuous variables. A linear regression model was fitted using tissue outcome as the dependent and age, gender, and predictors of tissue outcome as independent variables using \( P < 0.05 \) as retention criterion. Because tissue outcome metrics did not conform to normal distribution, they were each log-transformed before being introduced to the model. All categorical variables were entered as binary variables. Standard regression diagnostics were used to assess linear regression assumptions. There was no evidence of collinearity between the covariates or nonlinearity between the dependent variable and independent variable. All numeric variables were expressed as mean±SD or median (interquartile range [IQR]). A 2-tailed probability value of <0.05 was considered significant. Statistical analyses were performed using SPSS 11.5.

**Results**

A total of 756 consecutive with DWI and PWI within the first 12 hours of symptom onset were screened during the study period. Patients were excluded if they did not provide informed consent for follow-up imaging or failed to return back for a follow-up study (435 patients); were enrolled into a therapeutic trial or underwent intra-arterial thrombolysis (90 patients); and had extensive hemorrhagic conversion, massive brain edema, or hemianciectomy (28 patients). Of the remaining 203 patients, 62 (31%) did not have DWI/MTT mismatch according to the predefined criteria. Further analyses were restricted to the remaining 141 patients.

The study population consisted of 53 women and 88 men with a median (IQR) age of 71 (56 to 80) years. Initial and follow-up images were acquired after a median (IQR) of 5.3 (3.5 to 7.2) hours and 10 (6 to 33) days from stroke onset, respectively. Thirty-eight patients had follow-up studies performed after 30 days. The median (IQR) lesion volume was 14.7 mL (4.6 mL to 48.7 mL) on acute DWI and 34.5 mL (10.3 mL to 94.6 mL) on follow-up images. The median (IQR) PML was 16.4% (2.6% to 47.8%). PML increased by increasing age, admission plasma glucose level, admission National Institutes of Health Stroke Scale score, DWI lesion volume, follow-up lesion volume, MTT lesion volume, and by decreasing time from symptom onset to follow-up imaging (Table 1). The correlation between age and PML appeared to be driven by a steep increase in PML in patients >70 years as previously suggested.

There was no significant association between PML and gender. Nevertheless, women ≤70 years old were more likely to have smaller PML (\( P = 0.061 \)) as compared with their male counterparts, whereas there was no difference in PML between men and women >70 years (Table 2; Figure). The linear regression analysis showed that gender was not a predictor of PML (Table 3). Gender, however, was a significant predictor of PML (\( P = 0.027 \)) when age-by-gender interaction term was included into the linear regression model. The adjusted PML from the regression model was 0.44-fold (95% CI, 0.17 to 0.95; \( P = 0.033 \)) smaller in women younger than the group median (≥70 years old) as compared with men in the similar age strata. In contrast, in patients >70 years old, PML was 1.60-fold (95% CI, 0.90 to 2.81;
P/11005.091) higher in women as compared with men. Both gender and age-by-gender interaction remained significant when the regression model was repeated by using a forward stepwise selection approach including all variables presented in Table 1 as covariates. The results did not change when the regression model with PML was repeated after including the 28 patients who were initially excluded from analysis due to massive edema, extensive hemorrhagic conversion, or hemicraniectomy on follow-up imaging; assuming that the whole territory at risk underwent infarction in such patients (ie, PML was 100%), both gender (P/11005.039) and the interaction between age and gender (P/11005.030) were significant predictors of PML. Similarly, there was no change in the results when the 10 patients who had follow-up MRI performed by 3-T scanners were excluded (P for age-by-gender interaction 0.001). There were 4 women who were on hormone replacement therapy at the time of stroke onset. They had significantly lower PML when compared with the remaining 49 women (P/11005.046). There was no change in the results when these 4 patients were excluded from the multivariable analyses (P for age—by-gender interaction 0.031).

Fifty-one patients received intravenous thrombolytic treatment. The median PML was higher in treated patients as compared with those who were not treated (23% versus 11%, P/11005.063; Table 1). Patients who received intravenous thrombolytic treatment had more severe strokes at baseline (median National Institutes of Health Stroke Scale score, 14 in treated; 7 in untreated; P<0.001). The median PML was 14% (IQR, 6%–43%) in women and 25% (IQR, 6%–47%) in men among the 51 treated patients (P/11005.750) and 9% (IQR, 1%–26%) in women and 14% (IQR, 2%–49%) in men in the 90 untreated patients (P/11005.480).

**Discussion**

In this report of 141 patients, the relative percentage of initially ischemic tissue that eventually turned into infarction

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**Table 1. Univariate Associations Between Baseline Clinical and Imaging Variables and PML**

<table>
<thead>
<tr>
<th>Variable</th>
<th>PML Median (IQR) or Correlation Coefficient</th>
<th>P</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
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</tr>
<tr>
<td>Female (n=53)</td>
<td>12% (3%–55%)</td>
<td></td>
</tr>
<tr>
<td>Male (n=88)</td>
<td>19% (2%–46%)</td>
<td></td>
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<tr>
<td>Intravenous tissue plasminogen activator therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=51)</td>
<td>23% (6%–55%)</td>
<td></td>
</tr>
<tr>
<td>No (n=90)</td>
<td>11% (2%–36%)</td>
<td></td>
</tr>
<tr>
<td>CCS subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large artery atherosclerosis (n=36)</td>
<td>19% (4%–43%)</td>
<td></td>
</tr>
<tr>
<td>Cardioaortic embolism (n=64)</td>
<td>18% (5%–46%)</td>
<td></td>
</tr>
<tr>
<td>Other (n=17)</td>
<td>11% (0%–70%)</td>
<td></td>
</tr>
<tr>
<td>Undetermined (n=24)</td>
<td>9% (0%–59%)</td>
<td></td>
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<tr>
<td>Type of follow-up imaging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT (n=50)</td>
<td>22% (4%–59%)</td>
<td></td>
</tr>
<tr>
<td>MRI (n=91)</td>
<td>12% (3%–35%)</td>
<td></td>
</tr>
<tr>
<td>Age r</td>
<td>0.26</td>
<td>0.002</td>
</tr>
<tr>
<td>Admission mean blood pressure r</td>
<td>0.06</td>
<td>0.515</td>
</tr>
<tr>
<td>Admission plasma glucose level r</td>
<td>0.20</td>
<td>0.017</td>
</tr>
<tr>
<td>Admission NIHSS score r</td>
<td>0.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time from onset to initial imaging r</td>
<td>−0.14</td>
<td>0.095</td>
</tr>
<tr>
<td>Time from onset to follow-up imaging r</td>
<td>−0.23</td>
<td>0.006</td>
</tr>
<tr>
<td>Admission DWI lesion volume r</td>
<td>−0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Admission MTT volume r</td>
<td>−0.26</td>
<td>0.002</td>
</tr>
</tbody>
</table>

CCS indicates Causative Classification of Stroke; NIHSS, National Institutes of Health Stroke Scale.

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**Table 2. Tissue MRI Metrics According to Age and Gender**

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age ≤70 years</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lesion Volume, mL</td>
<td>22 (5–59)</td>
<td>9 (5–35)</td>
<td>41 (11–106)</td>
<td>16 (7–47)</td>
<td>18 (1–35)</td>
<td>7 (0–12)</td>
</tr>
<tr>
<td><strong>Age &gt;70 years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion Volume, mL</td>
<td>14 (4–41)</td>
<td>13 (4–62)</td>
<td>46 (8–98)</td>
<td>35 (13–130)</td>
<td>25 (3–56)</td>
<td>25 (8–87)</td>
</tr>
</tbody>
</table>

*P<0.05.
†P=0.061.
‡P=0.295.

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**Figure.** Percentage mismatch lost as a function of age and gender. The graph shows the individual data points (o), mean percentage mismatch lost for each age group (■: men; ●: women), and the corresponding SE bars. Note that percentage mismatch lost is less in women compared with men among patients ≤70 years old, whereas there is no difference in percentage mismatch lost between men and women >70 years old.
(PML) was similar between men and women with or without adjustment for important predictors of tissue outcome. However, there was an age-by-gender interaction; when age was considered in 2 strata (≤70 and >70 years), younger women had smaller PML (0.44-fold) compared with younger men, whereas older women had marginally larger PML (1.60-fold) compared with older men.

Our finding that there is a differential age-dependent worsening in tissue outcome in men and women coincides with evidence from observations on the relationship between gender and clinical outcome. It is known that premenopausal women are at lower risk of developing cardiovascular diseases, including stroke and vascular death, compared with age-matched men. The risk of stroke increases in postmenopausal women and becomes equal to that of men by the time women are approximately 65 to 75 years old. Mortality and functional outcome after stroke also shows age-dependent change between men and women; the US age-specific stroke mortality statistics demonstrate that women aged 45 to 74 years have a lower risk of stroke mortality compared with men, whereas no such survival advantage is observed for women after the age of 75 to 80 years. The mortality rates in older women even surpass the rates observed in their male counterparts.

Table 3. Multivariable Predictors of PML With or Without Including an Age-by-Gender Interaction

<table>
<thead>
<tr>
<th></th>
<th>Without Age-by-Gender Interaction</th>
<th>With Age-by-Gender Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>P</td>
</tr>
<tr>
<td>Age &gt;70 years</td>
<td>0.56</td>
<td>0.120</td>
</tr>
<tr>
<td>Female gender</td>
<td>-0.28</td>
<td>0.430</td>
</tr>
<tr>
<td>Age×gender interaction</td>
<td>. . .</td>
<td>. .</td>
</tr>
<tr>
<td>Admission NIHSS score</td>
<td>0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Admission MTT volume</td>
<td>0.002</td>
<td>0.477</td>
</tr>
<tr>
<td>Admission DW lesion volume</td>
<td>0.01</td>
<td>0.285</td>
</tr>
<tr>
<td>Admission plasma glucose level</td>
<td>0.002</td>
<td>0.634</td>
</tr>
<tr>
<td>Time to follow-up imaging</td>
<td>-0.03</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NIHSS indicates National Institutes of Health Stroke Scale.

The estrogen hypothesis is further supported by data from animal models of cerebral ischemia. Estrogen has been shown to exert potent effects on the vascular wall; it induces vasodilation through nitric oxide-dependent mechanisms or by directly acting on the vascular smooth muscle cells. In addition, estrogen has anti-inflammatory, antioxidant, anti-apoptotic, and neuroprotectant effects. It has been shown that the effect of gender on infarct size disappears after ovariectomy. It has also been reported that the size of the infarction increases with aging in female mice after middle cerebral artery occlusion; infarct volume is smaller in younger and larger in older female animals as compared with their male counterparts, a pattern that completely overlaps with the findings of current studies in humans.

This study is subject to certain limitations. The study population was selected based on the availability of baseline imaging within a relatively narrow time window (12 hours of stroke onset). This may have caused exclusion of patients with severe strokes who were not clinically stable enough for an early MRI, patients with mild stroke with delayed hospital admission, patients with grave short-term prognosis, and patients with contraindications for MRI. This might have led to a bias toward selection of a subset with a specific growth pattern. Nevertheless, the impact of this would not be clinically significant because this study represented a population in which infarct growth was the most clinically relevant. Second, follow-up images were obtained at different time points. This is inevitable in longitudinal research studies that are required to adjust their research priorities according to the medical condition and availability of patients. Infarct growth typically occurs within the first 48 hours of stroke onset but ischemic lesions continue to expand beyond this point because of vasogenic edema. It has been suggested that 30-day lesion volume is a reasonable approximation for final infarct volume. The median time to follow-up was 10 days in the current study. This might have caused overestimation of infarct growth because of contribution of vasogenic edema. Nevertheless, this would not be expected to change our results significantly because “time from onset to follow-up imaging” was taken into account in multivariable models as a covariate.

The clinical outcome after stroke is less favorable in older women compared with younger women and men. It has been suggested that limited access to primary stroke prevention and high prevalence of risk factors such as atrial fibrillation and metabolic syndrome in older women could explain the gender effect. Others have highlighted the importance of more indirect poststroke factors in older women such as social isolation, lower social status, issues with accessibility to health care, and frequent presence of comorbidities such as depression, all of which can impede access and response to rehabilitation after stroke. The current findings extend our knowledge on a differential age-by-gender effect in clinical outcome to the tissue level and add to the accumulated body of evidence that intrinsic mechanisms that govern the brain’s susceptibility to ischemia may also differ by age and gender. Further studies are needed to elucidate the biological mechanisms that modulate the interaction among age, gender, and tissue outcome in response to ischemia.
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Disclosures
None.

References
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