Prognostication of Recovery in Patients With Acute Ischemic Stroke Through the Use of Brain SPECT With Technetium-99m—Labeled Metronidazole

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Background and Purpose—We hypothesized that technetium-99m-ethylene dicysteine-metronidazole (^{99m}Tc-EC-MN) localizes to brain tissue that is hypoxic but viable. This study prospectively evaluated the relationship between neurological outcome and uptake of ^{99m}Tc-EC-MN in peri-infarcted regions of the brain.

Methods—Eight patients with acute ischemic stroke in the territory of the left middle cerebral artery underwent ^{99m}Tc-EC-MN and ^{99m}Tc-ethyl cysteinate dimer (ECD) brain SPECTs on the same day during the subacute stage (10.3±2.5 days). The infarct volumes from ^{99m}Tc-ECD images (IV_{ECD}), infarct volumes from diffusion-weighted MRI images (IV_{DW}), and hypoxic volume (HV) from ^{99m}Tc-EC-MN images were calculated. The net infarct volume (NIV_{ECD}) was defined as IV_{ECD} minus HV. The National Institutes of Health Stroke Scale scores were measured on admission and days 1, 3, 7, and 30.

Results—IV_{ECD} was greater than IV_{DW}. The lesion-to-normal count-density ratios of ^{99m}Tc-EC-MN ranged from 1.80 to 5.96. HV was 60.2±65.2 cm^3, and the mean percent HV was 24.5±28.1% of IV_{ECD}. NIV_{ECD} was 162.6±133.4 cm^3 and was significantly smaller than IV_{ECD}. NIV_{ECD} was significantly correlated with National Institutes of Health Stroke Scale score at 1 month and was a significant predictor of neurological deficit at 1 month.

Conclusions—^{99m}Tc-EC-MN brain SPECT can detect hypoxic tissue after acute ischemic stroke and, in combination with ^{99m}Tc-ECD brain SPECT, is useful in predicting neurological outcome in ischemic stroke patients. **(*Stroke*. 2003;34: 982-986.)**

Key Words: cerebral infarction ■ metronidazole ■ prognosis ■ technetium compounds ■ tomography, emission computed

The ischemic penumbra is an area of brain tissue with a level of blood flow near the threshold for maintenance of function and morphological integrity.[1] The penumbra is characterized by PET[2] as a region with reduced regional cerebral blood flow (rCBF), an increased oxygen extraction fraction, and relatively preserved oxygen consumption ($CMRO_2$). The penumbral tissue has the potential to recover and thus is a target for interventional therapy in acute ischemic stroke. However, after a few hours or days, the metabolism of glucose and oxygen may fall below values necessary for cell survival, resulting in the final transition from ischemia to infarction. Therefore, assays for oxygen insufficiency appear to be the simplest and most essential tools in the management of stroke.

Markers of hypoxic tissue have been tested for their ability to identify penumbral tissues.[3] PET with F-18 fluoromisonidazole (FMISO), a nitroimidazole derivative, was studied as an alternative, simple method for identifying penumbral tissues in patients with acute ischemic stroke; this method identified hypoxic tissues during the first 48 hours after stroke.[4,5]

Technetium-99m (^{99m}Tc) has favorable physical characteristics, has a low price, and is readily available, unlike fluorine-19. Recently, ^{99m}Tc-ethylene dicysteine-metronidazole (EC-MN) has been developed to assess the hypoxic components of cerebrovascular accident, myocardial infarction, and various tumors.[6] Similar to other nitroimidazole derivatives such as FMISO, metronidazole is trapped within hypoxic cells. Therefore, we hypothesized that ^{99m}Tc-EC-MN localizes to brain tissue that is hypoxic but viable after ischemic stroke and that the higher the uptake of ^{99m}Tc-EC-MN, the better the neurological outcome.

The aims of this study were as follows: (1) to determine whether brain SPECT with ^{99m}Tc-EC-MN can detect peri-

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infarct hypoxic tissues that are likely to represent the ischemic penumbra in patients with ischemic stroke, (2) to evaluate how long hypoxic tissues identified by ^{99m}Tc-EC-MN persist after acute ischemic stroke, and (3) to determine whether hypoxic tissue volumes correlate with clinical outcome.

Subjects and Methods

Subjects

We prospectively studied 8 consecutive patients (4 men, 4 women; mean age, 71±8 years) with acute ischemic stroke in the territory of the left middle cerebral artery who underwent brain MRI and received conservative therapy within 6 hours of the attack. Acute ischemic stroke was defined by (1) the presence of ≥1 neurological deficits such as hemiparesis, aphasia, or dysphasia, and (2) the finding of acute cerebral infarction on early MRI. Criteria for exclusion from entry or continuation in the study were previous stroke, primary intracerebral hemorrhage, infarctions involving the brain stem, lacunar infarctions, or any other factor that might result in a high risk of the patient defaulting from follow-up. The study was approved by the ethics committee at our hospital.

MR Imaging

All MRIs were performed with a 1.5-T whole-body superconducting imager (Signa Horizon, GE Medical Systems). The head coil was used. Sagittal T1-weighted images were used for localization. A spin-echo sequence (repetition time [TR]/effective echo time [TE]=500 ms/13 ms) was used for axial T1-weighted images.

Axial multisliced T2 fast-spin echo images were obtained with the following sequences: a TR/effective TE of 4000 ms/100 ms, an echo train length of 12, a matrix number of 256×192, a field of view of 14 mm, a slice thickness of 3 mm, a gap of 1 mm, and 2 excitations. Non–cardiac-gated single-shot echo-planar diffusion-weighted images (DWIs) were obtained with the following sequences: a TR/TE of 10 000 ms/100 ms, a matrix number of 128×128, and a field of view of 14 cm. We used oblique slices that were 4 mm thick with a 1-mm gap. Diffusion gradients were sequentially activated in each of the 3 principal anatomic axes to obtain DWIs that were sensitive to diffusion in the *x*, *y*, and *z* planes. A gradient strength that corresponded to a *b* value of 1000 s/mm^2 was used.

SPECT Imaging

^{99m}Tc-EC-MN and ^{99m}Tc-ethyl cysteinate dimer (ECD) brain SPECT images were acquired on the same day during the subacute stage (10.3±2.5 days; range, 6 to 14 days). One-day subsequent subtraction brain SPECT was performed with ^{99m}Tc-EC-MN and ^{99m}Tc-ECD through the use of a modification of the protocol of Song et al.[7]

The first SPECT scanning began 2 hours after injection of 1110 MBq of ^{99m}Tc-EC-MN synthesized by use of methods previously described by Yang et al.[6] Before the SPECT scanning was performed, an intravenous line was established in all subjects while they were lying down with their eyes open and ears unplugged in a quiet, dimly lit room. A dual-head rotating gamma camera (DST, SMV) equipped with a low-energy, high-resolution, parallel multihole collimator that peaks at 140 keV and has a symmetric 20% window was used to obtain tomographic images. Sixty-four projections of 40 seconds each over a 360° circular orbit were obtained. With no change made to the subject's head position, an intravenous injection of 110 MBq of ^{99m}Tc-ECD (Neurolite, DuPont Pharma) was given immediately after the first data acquisition was stopped. Five minutes later, a second projection data acquisition that had acquisition conditions identical to those of the first was begun.

To obtain ^{99m}Tc-ECD projection data, the first projection data were subtracted from the second SPECT data multiplied by 1.067, which was the correction coefficient for the decay of ^{99m}Tc between the first and second SPECT studies.

Tomographic images were reconstructed from the 3-dimensional 128×128×128 matrix in the transverse plane using backprojection algorithms with a Butterworth filter with an order of 5, a Nyquist frequency cutoff of 0.22, and a software zoom of 2. No attenuation correction was performed. Three sets of transverse, sagittal, and coronal slices, which covered the whole brain, were obtained for the ^{99m}Tc-ECD and ^{99m}Tc-EC-MN images.

Image Analysis

Postprocessing of MRIs was performed with software based on a homemade image analysis application (Chonnam National University Hospital). DWI volumes were measured from the maximum diffusion sensitivity isotropic image of the hyperintense infarct and the surrounding tissue. The edge of the infarct was identified visually, and regions of interest were outlined with a manual, pixel-wise method. The whole-brain volume and infarct volume (IV_{DW}) were calculated by summing the area of the region of interest and multiplying by the slice thickness plus the interslice gap. Known anatomic markings such as ventricles and large sulci were taken into account, although the outline bridged small cortical sulci in cortically based infarcts. A difference in lesion volumes of >10% was considered significant and was probably not caused by measurement error.

Each volume was calculated by drawing along the cortical boundary using irregular regions of interest in consecutive, transverse images. Pixel counts of the infarct areas and whole brain were measured on ^{99m}Tc-ECD images, and pixel counts of hypoxic areas were measured on ^{99m}Tc-EC-MN images. The infarct volume (IV_{ECD}) was defined as the volume of lesions showing perfusion that was <90% of that of the contralateral normal brain on ^{99m}Tc-ECD images. The hypoxic volume (HV) was defined as the volume showing an uptake of ^{99m}Tc-EC-MN that was greater than 30% of that of the contralateral normal brain. Each volume was calculated from the following equation: total volume=number of voxels×volume of voxel; voxel volume=1.97×1.97×3.45=13.389 mm^3. Net infarct volume (NIV_{ECD}) was defined as the infarct volume calculated from ^{99m}Tc-ECD brain SPECT minus the HV calculated from ^{99m}Tc-EC-MN brain SPECT.

Assessment of Neurological Status and Outcome

The severity of the patients' neurological deficits was assessed on admission and on days 1, 3, 7, and 30 with the National Institutes of Health Stroke Scale (NIHSS; scored from 31 to 0) of Goldstein et al.[8] Clinical outcome was measured on day 30 with the NIHSS.

Statistical Analysis

Statistical analyses were performed with the SPSS software package (version 10.0, SPSS Inc). Descriptive data are presented as mean±SD. Continuous variables, including each volume calculated from brain MRI and SPECT DWIs, were compared by use of Student's *t* test. The correlations between imaging findings and clinical outcome scales were evaluated with Spearman's rank correlation coefficient.

A multiple linear stepwise regression model was used to analyze the association between the dependent variable (NIHSS outcome at 1 month) and the independent variables (IV_{DW}, IV_{ECD}, NIV_{ECD}, and time from onset to SPECT imaging). Regression analysis was performed with a stepwise multiple linear regression procedure in which only the independent variables were included and retained in the regression model at a chosen 5% significant level. The difference was considered to be significant if $P<0.05$.

Results

Table 1 summarizes patient information for timing of SPECT imaging, IV_{DW}, IV_{ECD}, NIV_{ECD}, and changes in NIHSS score. The whole-brain volume and IV_{DW} calculated from DW MRI in all 8 patients were 1077.0±213.6 and 79.8±71.8 cm^3, respectively. The mean percent infarct volume was 7.5±6.7% (range, 1.0% to 17.7%) of the whole-brain volume. The whole-brain volume and IV_{ECD} calculated from perfusion

TABLE 1. Patient Information Showing IV_{DW}, Timing of SPECT, IV_{ECD}, NIV_{ECD}, and NIHSS Scores in 8 Patients With Acute Ischemic Stroke in the Territory of the Left Middle Cerebral Artery

					Brain SPECT									
			DW MRI		Time from Onset to SPECT Imaging, d	^{99m}Tc-ECD		^{99m}Tc-EC-MN				NIHSS		
Patient	Age, y	Sex	WB Volume, cm^3	Infarct Volume, cm^3		WB Volume, cm^3	Infarct Volume, cm^3	HV (cm^3)	HV/IV, %	L/N Ratio	Net Infarct Volume, cm^3	Day 1	Day 3	Day 7	Day 30
1	59	F	938.3	146.8	9	906.9	383.1	126.9	33.1	3.47	256.2	13	13	13	13
2	70	F	829.0	9.9	12	1011.9	114.0	3.3	2.9	5.09	110.7	5	14	14	14
3	63	M	964.1	103.6	6	1275.8	203.9	22.4	11.0	2.80	181.5	13	13	12	10
4	87	M	1038.3	32.6	8	1079.0	75.4	7.2	9.6	3.56	68.2	14	13	4	4
5	73	M	1190.5	211.2	11	946.2	580.5	139.9	24.1	1.84	440.7	20	20	16	15
6	75	F	1536.4	36.5	12	1057.6	149.2	22.8	15.3	3.08	126.4	7	7	5	4
7	68	M	1058.0	87.2	14	1232.8	164.4	147.9	90.0	5.96	16.5	4	2	1	1
8	70	F	1061.4	10.2	10	1002.0	111.2	10.8	9.7	1.80	100.4	14	14	12	9
Mean	70.6		1077.0	79.8	10.3	1064.0	222.7	60.2	24.5	3.45	162.6	11.3	12.0	9.6	8.8
SD	8.4		213.6	71.8	2.5	130.2	172.7	65.2	28.1	1.46	133.4	5.4	5.3	5.5	5.2

WB indicates whole brain; IV, infarct volume; and L/N, lesion-to-normal brain uptake ratio of ^{99m}Tc-EC-MN.

brain SPECT images with ^{99m}Tc-ECD were 1064.0±130.2 and 222.7±172.7 cm^3, respectively. The mean percent infarct volume was 22.1±19.2% (range, 7.0% to 61.3%) of the whole-brain volume. There was no significant difference between whole-brain volumes measured on DW MRI and brain SPECT images with ^{99m}Tc-ECD (P=0.916). However, IV_{ECD} was significantly greater than IV_{DW} (P=0.0274).

All 8 patients were studied 6 to 14 days (10.3±2.5 days) after the onset of stroke. At this time, they exhibited variable uptakes of ^{99m}Tc-EC-MN in the peripheries of the infarct areas and no uptake in the contralateral, normal hemispheres or in areas of old cerebral infarction on SPECT scan (Figure 1). The distribution and degree of uptake of ^{99m}Tc-EC-MN differed in various types of cerebral infarction. In embolic infarctions, a higher uptake of EC-MN was noted in most areas except the medial portion of the acute infarct area; this area was considered to be the infarct core in 2 patients (patient 7 and 1 patient not included in the study because of loss of neurological outcome). However, in the other 7 patients with thrombotic infarctions, focal, irregular uptake of ^{99m}Tc-EC-MN along the margin of the infarcted areas was observed. The infarct-to-normal count-density ratios for ^{99m}Tc-EC-MN ranged from 1.80 to 5.96 (3.45±1.46). The HV was 60.2±65.2 cm^3, and the mean percent volume was 24.5±28.1% (range, 2.9% to 90.0%) for IV_{ECD}. NIV_{ECD} indicated that the pure perfusion defect volume was 162.6±133.4 cm^3 and was significantly smaller than IV_{ECD} (P=0.035).

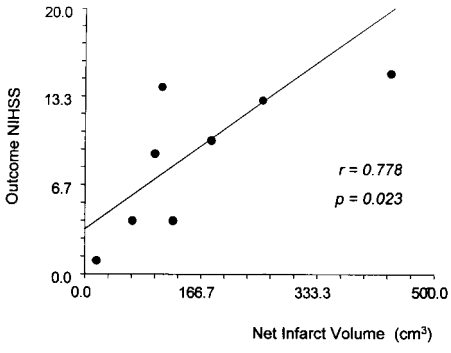

Figure 1. Positive correlation between net infarct volume during subacute stage and NIHSS outcome at 1 month.

Spearman's rank correlation test showed a strong positive correlation between NIHSS outcome at 1 month and NIV_{ECD} (r=0.778, P=0.023; Figure 2). This indicates that the smaller the volume of the perfusion defect, the better the neurological outcome. Multiple linear stepwise regression was used to determine the variables predicting neurological deficit at 1 month after stroke onset. The variable entered into the equation was NIV_{ECD} (P=0.035; Table 2), which provided independent prognostic information at 1 month. IV_{DW}, IV_{ECD}, HV, and the time from onset to SPECT imaging were not independent outcome predictors.

Discussion

In this prospective study using brain SPECT with ^{99m}Tc-EC-MN, we identified hypoxic tissue in patients with ischemic stroke during the subacute stage. Neurological outcome correlated with net perfusion defect volume on ^{99m}Tc-ECD brain SPECT, which is the whole perfusion defect volume minus the HV found on ^{99m}Tc-EC-MN.

In the human studies of Read et al,[4,5] who used PET with F-18 FMISO, peri-infarct hypoxic tissues were detected after acute ischemic stroke. Furthermore, autoradiography with labeled nitroimidazole derivatives revealed increased tracer uptake that was associated with histologically damaged areas and adjacent areas that appeared intact in animal models of cerebral ischemia.[9–11] These findings support our results using brain SPECT with ^{99m}Tc-EC-MN. The degree of distribution and uptake of ^{99m}Tc-EC-MN differed between types of cerebral infarction in our study. Although Read et al[4] did not

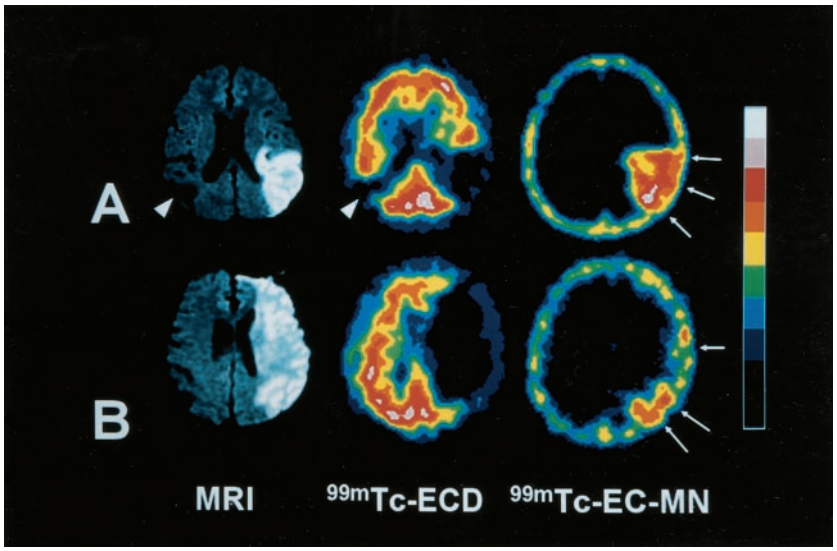

Figure 2. Two patients demonstrated large infarction areas on DW MRI and ^{99m}Tc-ECD brain SPECT. Patient 7 (A) showed an area of good uptake of ^{99m}Tc-EC-MN (white arrow) and had a 1-month NIHSS score of 1, despite the presence of an old infarction in the right parietal lobe (arrowhead) that showed no uptake of ^{99m}Tc-EC-MN. Patient 5 (B), on the other hand, showed an area of poor uptake of ^{99m}Tc-EC-MN (white arrow) and had a 1-month NIHSS score of 15.

comment on the distribution of hypoxic tissues in different types of cerebral infarction, similar distributions were observed in their cases.

Another study[10] of hypoxic marker trapping revealed that the ^{99m}Tc complex of a 2-nitroimidazole–derivatized propylene amine oxime is selectively retained in acutely ischemic brain but not in the ischemic infarct before disruption of the blood-brain barrier. However, a change in permeability of the blood-brain barrier occurs between the 24th hour and the third day after ischemia.[12] Because our study was performed during the subacute stage of cerebral infarction, we could not exclude that trapping of ^{99m}Tc-EC-MN might be influenced by disruption of the blood-brain barrier in ischemic tissue. Although no direct data concerning this issue are available, trapping of 2-nitronidazole derivatives is dependent on tissue hypoxia and is not associated with blood flow.[4,5,9–12]

Lythgoe et al[9] showed that ^{125}I-labeled iodoazomycin arabinoside was trapped in regions of moderately reduced perfusion but not in the infarct core, where perfusion was severely reduced, supporting the hypothesis that nitroimidazole labels penumbral peri-infarct tissues. As in the former study,[7] we performed subtraction brain SPECT using ^{99m}Tc-ECD and ^{99m}Tc-EC-MN on the same day and were able to compare rCBF and hypoxic tissues in the same brain cortex at the same time point. There was considerable discrepancy between ECD and EC-MN uptakes into affected sites. The regional distribution of ^{99m}Tc-ECD identifies areas of low flow but cannot differentiate between the infarct core and areas of ischemia. Shishido et al[13] reported that ECD failed to reveal reflow to infarcted regions in some patients with subacute infarction with uncoupled blood flow and metabolism and that the ECD images were similar to those of $CMRO_2$ measured by PET. Decreased retention of the tracer in hyperperfused infarct areas causes an underestimation of CBF on static ^{99m}Tc-ECD SPECT images.[14] This might indicate that a lower uptake of ECD is caused by a lower extraction coefficient in a low-flow ischemic situation. Therefore, our study revealed a discrepancy between ECD and EC-MN uptake to affected sites, despite significant uptake of ^{99m}Tc-EC-MN.

TABLE 2. Predictors of Neurological Deficit at 1 Month as Evaluated by the NIHSS Score

Predictor	β	P	R^2	F
Net infarct volume	0.029	0.035	0.551	7.352*

The dependent variable was NIHSS outcome. The independent variables were infarct volumes calculated from DW MRI and brain SPECT with ^{99m}Tc-ECD, HV calculated from brain SPECT with ^{99m}Tc-EC-MN, and net perfusion defect volume (ECD−MN volume). β indicates unstandardized regression coefficients.
*P=0.035.

How long does hypoxic tissue persist after stroke? In the study of Read et al,[4] F-18 FMISO trapping was detected in 9 of 13 patients 6.25 to 42.5 hours after stroke onset, but hypoxic tissues did not persist into the subacute phase of stroke (6 to 11 days). The latter result is not consistent with our findings because the infarcted areas of all patients showed variable uptakes of ^{99m}Tc-EC-MN. Tissue is affected by misery perfusion within 4 days of acute stroke in 45% to 57% of cases studied.[15] With a few exceptions, these tissues suffer progressive metabolic derangement and become necrotic during the subsequent 2 weeks. Marchal et al[16] found that penumbral tissues might make up as much as 50% of the final volume of the infarct during the first 17 hours after stroke. The bulk of these tissues, which may persist for as long as 48 to 72 hours after stroke, demonstrates progressive metabolic derangement and becomes incorporated into the final infarction volume. In some patients, a significant volume of misery-perfused tissue survives the ischemic process, and patients have a better neurological outcome when a larger volume of the penumbra survives the ischemic episode. In addition, studies[17,18] using ligand for cerebral benzodiazepine receptors demonstrated the uptake of iomazenil into subacute or chronic cerebral infarctions, suggesting the presence of actual viable neurons in the peri-infarcted area. We suggest that 2 factors influence the fate of tissues suffering from misery perfusion. The first factor is spontaneous reperfusion, which occurs within the first week of cerebral infarction and is associated with clinical improvement in only 2% of

patients.[19] The other factor is collateral growth and angiogenesis around the cortical stroke area. Wei et al[20] showed that arteriolar collateral growth and new capillaries restore perfusion to the ischemic border 30 days after ministroke and are able to support long-term functional recovery.

Relative or absolute rCBF maps fail to distinguish between the infarct core and ischemic penumbra and have limited value in the late diagnosis or prognosis of recovery in patients with ischemic stroke. However, when rCBF is measured in combination with $CMRO_2$ or DW MRI, it can reveal areas of misery perfusion that may proceed to infarction. Therefore, this may prove useful in classifying areas of recoverable tissue.[21] In our study, only NIV_{ECD} correlated with NIHSS outcome at 1 month and provided independent prognostic information at 1 month.

Despite the small number of patients, the data from brain SPECT with ^{99m}Tc-EC-MN in patients during the subacute stage of cerebral infarction are consistent with regard to neurological outcome. Further study using an established method for identifying the penumbra such as PET measurements of rCBF, $CMRO_2$, and oxygen extraction fraction is needed to evaluate the viability of brain tissues showing increased uptake of ^{99m}Tc-EC-MN in or around the infarcted areas on ^{99m}Tc-ECD brain SPECT and DWI; such studies should include patients with acute ischemic stroke.

Because ^{99m}Tc has favorable physical characteristics, is readily available and is low-priced, unlike fluorine-19. ^{99m}Tc-EC-MN can easily be used to identify hypoxic tissue in patients with ischemic stroke. On the basis of our observation of hypoxic tissues during the subacute phase after stroke, a rigid time window for stroke therapy may not be appropriate, as previously reported.[22] A certain patient may or may not have salvageable tissue that permits them to benefit from tissue-rescue therapies. In those who do, the duration of penumbral survival is likely to vary. Decisions about therapy may need to be individualized and based on some assessment of the amount of viable tissue present

In conclusion, ^{99m}Tc-EC-MN uptake in peri-infarcted areas suggesting the presence of actual viable neurons was observed during the subacute stage of cerebral infarction. Smaller-volume perfusion defects were associated with better neurological outcomes. ^{99m}Tc-EC-MN brain SPECT, combined with ^{99m}Tc-ECD brain SPECT, was useful in predicting neurological outcome in patients with ischemic stroke.

Acknowledgments

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