
Correlation of ^{99m}Tc -DTPA SPECT of the Blood–Brain Barrier with Neurologic Outcome After Acute Stroke

Mordechai Lorberboym, MD¹; Yair Lampl, MD²; and Menahem Sadeh, MD²

¹Department of Nuclear Medicine, Sackler Faculty of Medicine, Edith Wolfson Medical Center, Tel Aviv University, Tel Aviv, Israel; and ²Department of Neurology, Sackler Faculty of Medicine, Edith Wolfson Medical Center, Tel Aviv University, Tel Aviv, Israel

We conducted a study on humans to determine whether quantitative evaluation of blood–brain barrier (BBB) breakdown using the ^{99m}Tc -diethylenetriaminepentaacetic acid (^{99m}Tc -DTPA) SPECT technique at the peak time of stroke evolution can predict neurologic outcome. **Methods:** Thirty consecutive patients with acute stroke of the middle cerebral artery occurring >24 h and <48 h before admission were included in the study. Each patient underwent a complete neurologic examination according to the Scandinavian stroke score at 72 h after the stroke (S1) and again at 30 d (S2). The difference between initial and late scores was calculated (ΔS) and used to evaluate the change in neurologic status. A CT scan was obtained on all patients to determine the volume of stroke. The integrity of the BBB was evaluated using ^{99m}Tc -DTPA brain SPECT. A quantitative index of BBB disruption was defined as the ratio of the mean counts/pixel in the infarcted region compared with the mean counts/pixel in the contralateral nonaffected hemisphere. SPECT perfusion imaging was also performed with ^{99m}Tc -hexamethylpropyleneamine oxime (^{99m}Tc -HMPAO) at 24 h after the ^{99m}Tc -DTPA study. The relative perfusion in the infarct region was expressed as the percentage of contralateral perfusion. **Results:** The mean ^{99m}Tc -DTPA disruption index was 6.8 ± 6.9 (range, 1–26.2). Seven patients (23%) had no BBB disruption. Statistical analysis showed that the disruption index was negatively correlated with ΔS ($r = -0.423$, $P < 0.02$). A disruption index of <2.5 was associated with a significantly better neurologic outcome (mean ΔS , 17.5 ± 9.5) compared with patients with an index of >2.5 (mean ΔS , -0.85 ± 4.97 , $P < 0.0001$) with a sensitivity of 95% and a specificity of 89%. S2 was significantly correlated with S1 ($r = 0.738$, $P < 0.001$) and with ΔS ($r = 0.656$, $P < 0.001$). Perfusion abnormalities on the ^{99m}Tc -HMPAO SPECT studies ranged between 12% and 90% (mean, $37.6\% \pm 17.8\%$) compared with those on the contralateral nonaffected side. No correlation was found between ^{99m}Tc -HMPAO uptake and ΔS , infarct volume by CT, or disruption index. The CT volume measurements were negatively correlated with S2 ($r = -0.560$, $P < 0.004$) but not with ΔS . **Conclusion:** ^{99m}Tc -DTPA SPECT of the BBB combined with

quantitative analysis in patients with acute stroke is significantly related to clinical outcome, with a distinct prognostic cutoff threshold of 2.5. The use of this radionuclide brain SPECT technique represents a unique application of conventional non-diffusible brain agents.

Key Words: stroke; blood–brain barrier; ^{99m}Tc -DTPA; ^{99m}Tc -HMPAO; SPECT

J Nucl Med 2003; 44:1898–1904

Stroke is the third most common cause of death in the United States and a major cause of long-term disability (1). It is difficult to predict the neurologic outcome in the acute phase of cerebral ischemic strokes. Studies on animal models have shown that reduced blood flow to the brain can alter the blood–brain barrier (BBB) permeability and regulatory transport functions. The increased stress on the endothelium of the ischemically injured cells results in leakage of serum proteins and intracellular substances into the extracellular space. This mechanism is responsible for edema fluid accumulation in exacerbated ischemic brain edema (2–4). Studies on rats showed an initial, acute disruption of the BBB between 3 and 5 h after occlusion of the middle cerebral artery (MCA) and a later, more widespread increase in regional BBB permeability at 48 h (2). The volume of infarcted tissue was significantly correlated with the second step of the BBB breakdown (2,3).

Only a few studies have been designed in humans to compare the degree of BBB changes and the neurologic outcome after a stroke (5,6). These studies, however, demonstrated inconsistent correlation between infarct size and BBB disturbances.

Imaging studies have an important role in assessing the location and extent of the stroke, determining tissue viability, patient prognosis, and clinical management. CT can differentiate ischemic from hemorrhagic infarction and can identify other sites of intracranial bleeding. CT can also demonstrate mass effect after a stroke. However, CT has a limited role in the acute and subacute stages of the infarct in

Received Mar. 20, 2003; revision accepted Aug. 14, 2003.
For correspondence or reprints contact: Mordechai Lorberboym, MD, Department of Nuclear Medicine, Edith Wolfson Medical Center, Holon, 58100 Israel.
E-mail: mvlorber@internet-zahav.net

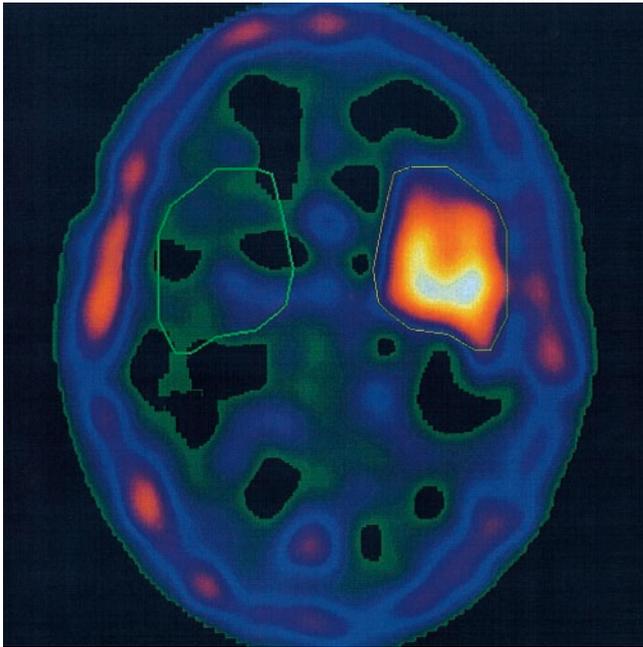


FIGURE 1. Tracing of ROI around infarct zone and contralateral hemisphere for calculations of ^{99m}Tc -DTPA index.

predicting the severity of neurologic deficits. Using MRI for this purpose is expensive and has practical limitations, considering that many of the elderly population have metal implants, such as pacemakers, which are not suitable for MRI. Although MRI in the first few hours after a stroke may predict further lesion growth in hyperacute stroke patients (7), most patients arrive at the hospital too late to receive the maximum benefit from emerging stroke therapies (8).

SPECT using perfusion agents (e.g., hexamethylpropyl-eneamine oxime [HMPAO] or ethylcysteinate dimer [ECD]) does not easily differentiate irreversibly compromised tissue from ischemic but viable tissue, because it does not directly assess neuronal function. Most PET studies in stroke aimed to visualize changes in blood flow and energy metabolism and found no correlation between the accumulation of radioactive tracers and the breakdown of the BBB (9).

^{99m}Tc -Diethylenetriaminepentaacetic acid (^{99m}Tc -DTPA) brain scintigraphy is the technique of choice for the assessment of BBB disruption. It has been used in the past to localize areas within the cranium that had been disrupted by infection, neoplasms, trauma, or stroke (10,11), but it has not been used previously to assess prognosis.

We conducted a study on humans to determine whether quantitative evaluation of BBB breakdown using the ^{99m}Tc -DTPA SPECT technique at the peak time of stroke evolution can predict neurologic outcome. The results were compared with the findings of cerebral perfusion studies using ^{99m}Tc -HMPAO SPECT and with concurrent CT volume measurements.

MATERIALS AND METHODS

Patients

Thirty consecutive patients (18 men, 12 women; mean age, 63 y; range, 38–87 y) were included in the study. Inclusion criteria were acute nonhemorrhagic stroke in the MCA territory occurring >24 h before admission (to avoid patients with transient ischemic attacks) and <48 h after the event with exact data on the onset of stroke. We excluded patients with other diseases of the central nervous system, including vascular malformations, tumors, multiple sclerosis, infectious diseases of the central nervous system, migraine headaches, and previous head injury. All patients were treated with 325 mg aspirin on admission, according to the guidelines of the international stroke trials (International Stroke Trial and Chinese Acute Stroke Trial) (12,13).

Each patient underwent a complete neurologic examination, including scoring of neurologic deficits according to the Scandinavian stroke score (SSS) (14). The scoring of the neurologic status was performed on admission, 3 d after onset of stroke (S1), and again at 30 d (S2). Day 3 was chosen for evaluation to demonstrate the BBB disruption intensity at the peak time of the deterioration phase, which usually occurs between days 2 and 4 (15). The difference between early (S1) and late (S2) scoring was calculated (ΔS) and used to evaluate changes in neurologic status.

CT was performed with and without contrast material in all patients using a Twin Flash scanner (Elscent). The CT scan was obtained 72 ± 6 h after infarction and was used to determine the volume of stroke by manual tracing of the infarct perimeter, as described (16).

^{99m}Tc -DTPA SPECT

The integrity of the BBB was evaluated using ^{99m}Tc -DTPA brain scintigraphy at 48–72 h after the stroke. Each patient received 740 MBq ^{99m}Tc -DTPA and a SPECT study was performed 2 h later. A dual-head γ -camera (Elscent-Helix) equipped with a pair of low-energy, high-resolution collimators was used. Images were acquired

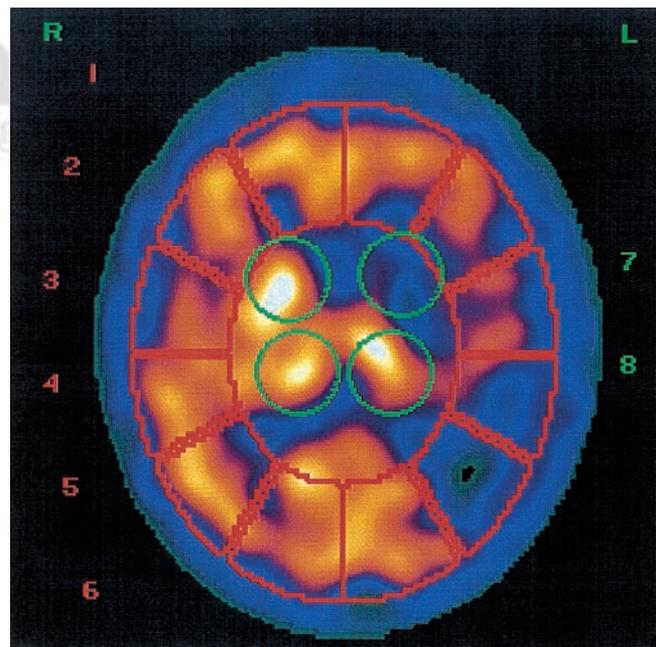


FIGURE 2. ROI analysis for determination of perfusion deficits at level of basal ganglia (left MCA infarct).

in a 128 × 128 matrix at 6° angular steps, 40 s each step. Transaxial, coronal and sagittal slices 1-pixel thick were reconstructed using a third-order Metz filter set to 12-mm full width at half maximum.

The transaxial slice with the most extensive ^{99m}Tc-DTPA uptake was selected, and a region of interest (ROI) was traced around the ^{99m}Tc-DTPA activity. An ROI of similar size and shape was drawn on the opposite side in a corresponding location (Fig. 1). A quantitative index of BBB breakdown (disruption index) was defined as the ratio of the mean counts/pixel in the infarcted region compared with the mean counts/pixel in the contralateral nonaffected hemisphere.

^{99m}Tc-HMPAO SPECT

SPECT perfusion imaging was performed 24 h after the ^{99m}Tc-DTPA SPECT study. Residual activity from the ^{99m}Tc-DTPA study was checked in every patient and found to be negligible, consistent with a lack of cerebral trapping of ^{99m}Tc-DTPA and prompt renal clearance. The perfusion study was performed 15–30 min after injection of 740 MBq ^{99m}Tc-HMPAO. One hundred twenty frames of 15 s were acquired using a circular rotation mode

into a 128 × 128 image matrix. The images were prefiltered using a Butterworth filter (cutoff frequency, 0.2 cycle/cm; power factor, 5). Transaxial, coronal, and sagittal slices 1-pixel thick were reconstructed. Attenuation correction was performed based on an automated ellipse determination with a constant linear attenuation coefficient of 0.11 cm⁻¹.

Quantitative evaluation of SPECT images was performed using computer-assisted sector analysis on all patients. Six representative transaxial slices parallel to the orbitomeatal line were selected and 6 symmetric pairs of ROIs were placed on both sides of the cerebral cortices, based on 30° angular separation. Manually drawn ROIs were used to define the head of the caudate nuclei and the thalami. The ratio of the average number of counts/pixel in the lesion to the average number of counts/pixel in the contralateral nonaffected area was obtained for all patients and expressed as the percentage of contralateral perfusion (Fig. 2).

Statistical Analysis

Data were analyzed using SPSS 9.0 Statistical Analysis Software (SPSS Inc.). Continuous data (age measurements) were ex-

TABLE 1
Clinical and Imaging Scores in Patients with Acute Stroke

Patient no.	Age (y)	Sex	Brain territory*	^{99m} Tc-DTPA ratio	^{99m} Tc-HMPAO† (%)	Score			Infarct volume (cm ³)	^{99m} Tc-DTPA		
						S1	S2	Δ S		>2.5	<2.5	Δ S <2.5
1	70	M	L MCA	11.1	40	19	20	1	NA	11.1		
2	38	F	R MCA	12	47	9	12	3	140	12		
3	67	M	L MCA	22.8	41	13	16	3	34	22.8		
4	68	M	L MCA	2.1	43	20	42	22	65		2.1	22
5	77	F	L MCA	26.2	45	25	27	2	0	26.2		
6	59	F	L MCA	1	15	30	47	17	NA		1	17
7	73	F	L MCA	15	41	4	4	0	35	15		
8	70	M	L MCA	1	12	13	47	34	12		1	34
9	60	M	R MCA	1	20	25	47	22	NA		1	22
10	39	M	R MCA	2.5	52	25	45	20	12		2.5	20
11	73	M	L MCA	3.6	25	47	49	2	105	3.6		
12	65	F	L MCA	6.5	62	13	14	1	265	6.5		
13	38	M	R MCA	2.2	37	17	40	23	0		2.2	23
14	87	F	R MCA	4.5	41	4	4	0	297	4.5		
15	45	M	L MCA	5.3	25	8	0	-8	404	5.3		
16	52	F	R MCA	10.5	35	10	13	3	94	10.5		
17	50	M	L MCA	3.5	90	10	8	-2	268	3.5		
18	70	F	L MCA	12	39	18	18	0	87	12		
19	53	M	L MCA	3.6	NA	12	12	0	4	3.6		
20	74	F	L MCA	9.6	NA	18	0	-18	129	9.6		
21	50	M	L MCA	1	43	14	27	13	150		1	13
22	73	M	R MCA	6.8	45	16	12	-4	NA	6.8		
23	78	F	R MCA	5.2	21	22	20	-2	150	5.2		
24	58	M	L MCA	3.1	14	23	23	0	NA	3.1		
25	56	M	L MCA	21	21	51	47	-4	24	21		
26	77	M	R MCA	1	50	50	50	0	10		1	0
27	67	F	R MCA	5.2	NA	14	16	2	115	5.2		
28	73	F	L MCA	3.2	32	9	13	4	64	3.2		
29	63	M	L MCA	1	65	8	26	18	197		1	18
30	70	M	R MCA	1	15	8	14	6	NA		1	6

*Territory of perfusion abnormality.

†Percentage reduction in perfusion (infarct zone).

NA = not applicable.

pressed as mean \pm SD and their distributions were tested for normalcy using the Kolmogorov–Smirnov test. All were found to have distributions not significantly deviating from normal. Categorical data (sex) were presented as frequency counts.

Pearson correlation analysis was used to describe associations between measurements. An iterative receiver-operating-characteristic analysis was performed to locate the ^{99m}Tc -DTPA cutoff point bearing the highest discriminating value. The iteration stepped on all possible cutoff points. The results indicated that the maximum area under the curve is seen at a cutoff point of 2.5. The cutoff was used to dichotomize ^{99m}Tc -DTPA, and ΔS was compared by this dichotomized ^{99m}Tc -DTPA variable using a *t* test for independent samples.

All tests were considered significant at $P < 0.05$ (2 tailed).

RESULTS

Nineteen of 30 patients had a left MCA infarct, and 11 patients had a right MCA infarct (Table 1).

The mean ^{99m}Tc -DTPA uptake ratio was 6.8 ± 6.9 (range, 1–26.2). Seven patients (23%) had no BBB disruption (uptake ratio, 1). Statistical analysis showed that the disruption index was negatively correlated with ΔS ($r = -0.423$, $P < 0.02$). A plot of the change in the stroke scale compared with the ^{99m}Tc -DTPA ratio is presented in Figure 3. A disruption index of <2.5 (10 patients, 33%) was associated with a significantly better neurologic outcome (mean ΔS , 17.5 ± 9.5) compared with patients with a ratio of >2.5 (mean ΔS , -0.85 ± 4.97 , $P < 0.0001$) with a sensitivity of 95% and a specificity of 89% (Fig. 4). The only patient with no BBB disruption and no clinical improvement (patient 26) had very mild abnormalities on admission (SSS, 50).

S2 was significantly correlated with S1 ($r = 0.738$, $P < 0.001$) and with ΔS ($r = 0.656$, $P < 0.001$). Perfusion abnormalities on the ^{99m}Tc -HMPAO SPECT studies ranged between 12% and 90% (mean, $37.6\% \pm 17.8\%$) compared with those of the contralateral nonaffected side. No correlation was found between ^{99m}Tc -HMPAO uptake and ΔS , infarct volume by CT, or disruption index. Figure 5 shows images of a patient with no evidence of BBB disruption, and Figure 6 shows images of a patient with extensive disruption of the BBB.

The CT volume measurements were negatively correlated with S2 ($r = -0.560$, $P < 0.004$) but not with ΔS .

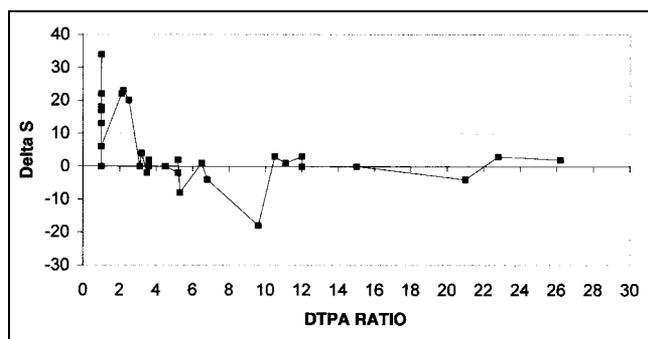


FIGURE 3. Plot compares change in stroke scale with increasing ^{99m}Tc -DTPA ratio.

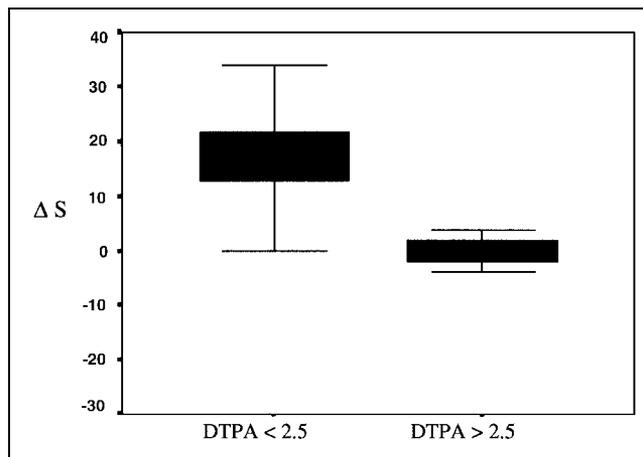


FIGURE 4. Distribution of ΔS values for ^{99m}Tc -DTPA index <2.5 and >2.5 .

DISCUSSION

The BBB is a highly selective barrier that prevents the passage of many substances from the blood into extracellular fluid of the brain, or into the cells, and vice versa. The data presented in our study show that measurement of the degree of BBB disruption at the peak of edema formation can be used efficiently and economically in humans as a marker of the neurologic status of the patients, and it may determine the late neurologic and functional outcome. Although thrombolytic therapy may be effective in selected patients if performed within 3–6 h after the onset of symptoms (17), most patients arrive at the hospital too late to benefit from thrombolysis. Less than 40% of patients may arrive within 24 h of the onset of symptoms (8). It appears from our study that changes in BBB permeability carry significant prognostic information beyond the hyperacute stage of the infarct.

^{99m}Tc -DTPA is a nondiffusible tracer for evaluation of BBB permeability, similar to ^{99m}Tc -pertechnetate and ^{99m}Tc glucoheptonate. ^{99m}Tc -DTPA brain scintigraphy has been used in the past to detect brain infarcts as well as brain metastases (10,11). It was used together with ^{111}In -octreotide scintigraphy to differentiate meningiomas from other central nervous system tumors (10), and it was also reported to detect multifocal opportunistic intracranial infection in AIDS patients (18). We have noticed in our study that areas of intense ^{99m}Tc -DTPA uptake are associated with a more severely damaged tissue. A disruption index threshold of <2.5 was a good predictor of favorable clinical outcome. In contrast, patients with an index of >2.5 had a significantly worse prognosis. Interestingly, a significant proportion of patients with stroke (23%) had no gross evidence of BBB disruption, all of them with very good prognosis.

Many experimental studies on animal models were performed to define the effect of BBB disruption in acute

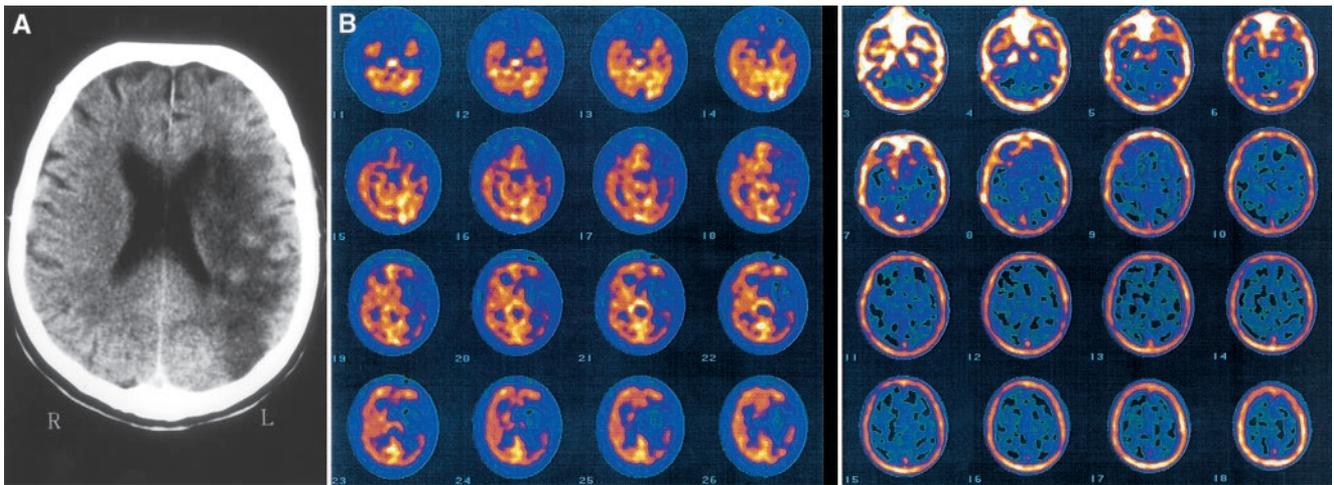


FIGURE 5. Patient 29: 63-y-old woman with extensive stroke in territory of left MCA. (A) Transaxial CT slice. (B) Transaxial SPECT perfusion slices (left) show diminished perfusion in left temporoparietal regions (perfusion index, 65%) and transaxial slices from ^{99m}Tc -DTPA SPECT (right) show normal distribution of ^{99m}Tc -DTPA (disruption index, 1). Patient improved significantly on follow-up (Δ S value, 18).

stroke (2–4). Studies in rats demonstrated preservation of the BBB for the first 2–3 h after MCA occlusion and progressive disruption after the first 6 h (2). Siegel et al. (19) used a multiple-tracer assessment of ischemic brain injury in rats, including ^{99m}Tc -pertechnetate. He proposed that abnormal molecular permeability of the BBB most likely results from damage to the endothelial cells or the intercellular junctions of the capillaries.

Only a few studies have been designed in humans to compare the degree of BBB changes and the neurologic outcome after a stroke. The most significant stage of BBB breakdown occurs in humans within 48–72 h after infarction and is accompanied by increased brain edema volume. One study that examined cerebrospinal fluid and serum

albumin ratios as a parameter for the degree of BBB opening found no difference between patients with a large or with a small infarct (6).

CT is used to detect hemorrhage and mass effect after a stroke; however, it is not sensitive at detecting brain ischemia in the acute stage of an infarct (20). MRI is more sensitive than CT, but it may fail to detect hyperacute ischemia (21,22). Perfusion-weighted MRI and diffusion-weighted MRI have been widely used to detect and quantify ischemia in experimental models (23). Diffusion-weighted MRI is sensitive to the translational motion of water protons and provides an image of apparent diffusion coefficients (ADCs). A mismatch between diffusion- and perfusion-weighted MRI is thought to define tissue at risk of infarct-

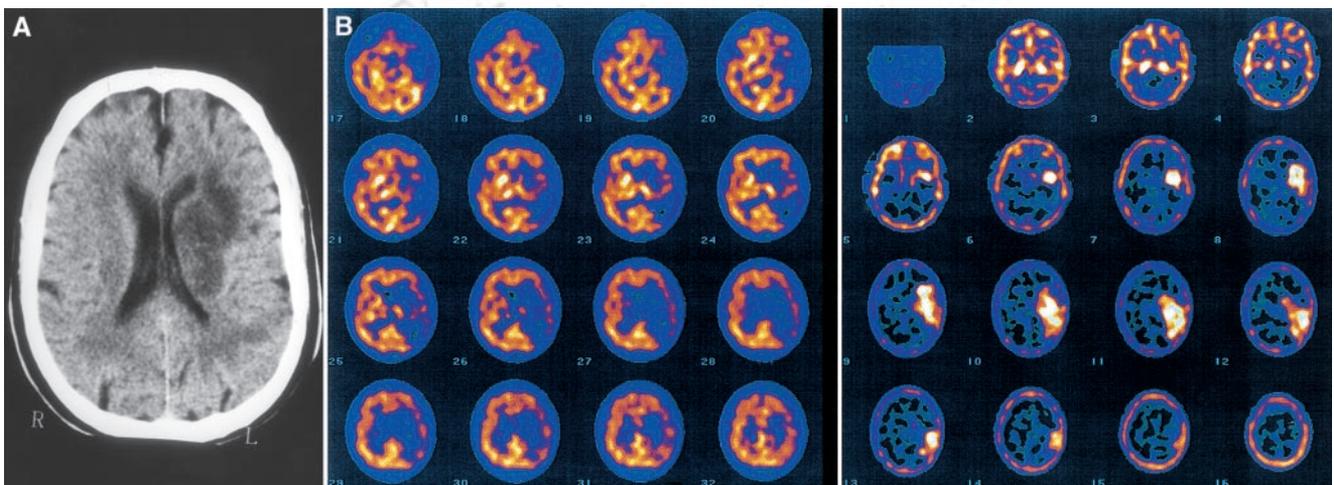


FIGURE 6. Patient 7: 59-y-old woman with stroke in territory of left MCA. (A) Transaxial CT slice. (B) Transaxial SPECT perfusion slices (left) show diminished perfusion in left temporoparietal regions (perfusion index, 41%) and transaxial slices from ^{99m}Tc -DTPA SPECT (right) show intensely increased ^{99m}Tc -DTPA uptake in same region (disruption index, 15). Patient showed no improvement on follow-up (Δ S value, 0).

tion. This concept is based on the assumption that diffusion slowing of and decreases in the ADC serve as an indicator of tissue proceeding to infarction. However, ADC decreases do not reliably indicate tissue infarction (24), and diffusion abnormalities probably correspond to early changes of both reversible and irreversible ischemia (25). Moreover, MRI is expensive and has practical limitations in many patients with surgical implants and pacemakers, whereas ^{99m}Tc -DTPA SPECT is more cost-effective and is easy to perform.

Most PET studies in stroke aimed to visualize changes in blood flow and energy metabolism. Marchal et al. (26) used PET with combined imaging of cerebral blood flow (CBF) and oxygen consumption to distinguish 3 different uptake patterns after the first MCA stroke, each associated with a different outcome.

SPECT perfusion imaging using HMPAO or ECD is very sensitive in detecting hypoperfusion after acute stroke. However, the hypoperfused tissue detected by lipophilic SPECT tracers is heterogeneous and contains irreversibly damaged, infarcted tissue and also dysfunctional viable tissue. Shimosegawa et al. (27) studied CBF early after the onset of cerebral infarction compared with CT findings. They concluded that a decrease of approximately 40% in CBF appears to represent the borderline between reversible and irreversible structural brain damage. However, there are drawbacks in estimating quantitative CBF, including postischemic hyperemia, which may interfere with quantitative analysis. In our study, the infarct zone showed a wide range of diminished perfusion (12%–90%) and no significant correlation with the clinical or computed variables.

The lack of correlation between ^{99m}Tc -HMPAO uptake and other stroke variables in our study deserves special attention. Our perfusion studies were not performed in the hyperacute stage (typically within 6 h after onset of the stroke) and, consequently, they may not have the predictive value that has been seen in early ^{99m}Tc -HMPAO studies. Additionally, it has already been demonstrated that astrocytes might constitute a prominent site of ^{99m}Tc -HMPAO retention and most likely contribute significantly to the SPECT signal (28). It was shown in a stroke model that both infarct volume and BBB disruption were greater in a reperfusion group compared with that of a permanent occlusion group (3,4), suggesting that brain infarct and BBB disruption are exacerbated after reperfusion following brain ischemia. These findings may explain in part the lack of correlation between perfusion imaging (as portrayed by ^{99m}Tc -HMPAO fixation) and cellular integrity (BBB disruption) in ischemic strokes. Other specific alterations in glial cell metabolism may contribute as well to flow-independent changes that may occur in the BBB (28).

CONCLUSION

Our data show that ^{99m}Tc -DTPA SPECT of the BBB combined with quantitative analysis in patients with acute

stroke is significantly related to clinical outcome, with a distinct prognostic cutoff threshold of 2.5. This technique may identify a subpopulation of patients at increased risk of neurologic deterioration and it may assist in the follow-up and management of patients with acute stroke. Further research is necessary to confirm these findings in a larger cohort of patients and to investigate the long-term association of BBB disruption and cerebrovascular stroke.

REFERENCES

1. Peters KD, Kochanek KD, Murphy SL. Deaths: final data for 1996. *Natl Vital Stat Rep.* 1998;47:1–100.
2. Belayev L, Busto R, Zhao W, Ginsberg MD. Quantitative evaluation of blood brain barrier permeability following middle cerebral artery occlusion in rats. *Brain Res.* 1996;739:88–96.
3. Kuroiwa T, Shibutani M, Okeda R. Blood-brain barrier disruption and exacerbation of ischemic brain edema after restoration of blood flow in experimental focal cerebral ischemia. *Acta Neuropathol.* 1988;76:62–70.
4. Yang BY, Betz AL. Reperfusion-induced injury to the blood-brain barrier after middle cerebral artery occlusion in rats. *Stroke.* 1994;25:1658–1664.
5. Hornig CR, Busse O, Buettner T, Dorndorf W, Agnoli A, Akengin Z. CT contrast enhancement on brain scans and blood-CSF barrier disturbances in cerebral ischemic infarction. *Stroke.* 1985;16:268–273.
6. Al-Kassab S, Skyhoj-Olsen T, Skriver EB. Blood-brain barrier integrity in patients with cerebral infarction investigated by computed tomography and serum-CSF-albumin. *Acta Neurol Scand.* 1981;64:438–445.
7. Fiehler J, von Bezold M, Kucinski T, et al. Cerebral blood flow predicts lesion growth in acute stroke patients. *Stroke.* 2002;33:2421–2425.
8. Smith MA, Doliszny KM, Shahar E, McGovern PG, Arnett DK, Luepker RV. Delayed hospital arrival for acute stroke: the Minnesota Stroke Survey. *Ann Intern Med.* 1998;129:190–196.
9. Stevens H, Jansen HM, De Reuck J, et al. ^{55}Co (Co) as a PET-tracer in stroke, compared with blood flow, oxygen metabolism, blood volume and gadolinium-MRI. *J Neurol Sci.* 1999;171:11–18.
10. Barth A, Haldemann AR, Reubi JC, et al. Noninvasive differentiation of meningiomas from other brain tumours using combined ^{111}In -octreotide/ ^{99m}Tc -technetium-DTPA brain scintigraphy. *Acta Neurochir Wien.* 1996;138:1179–1185.
11. Inoue Y, Momose T, Machida K, et al. Delayed imaging of ^{99m}Tc -DTPA-HSA SPECT in subacute cerebral infarction. *Radiat Med.* 1993;11:214–216.
12. The International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19,435 patients with acute ischaemic stroke—International Stroke Trial Collaborative Group. *Lancet.* 1997;349:1569–1581.
13. CAST: randomised placebo-controlled trial of early aspirin use in 20,000 patients with acute ischaemic stroke—CAST (Chinese Acute Stroke Trial) Collaborative Group. *Lancet.* 1997;349:1641–1649.
14. Scandinavian Stroke Study Group. Multicenter trial of hemodilution in ischemic stroke: background and study protocol. *Stroke.* 1985;16:885–890.
15. Hacke W, Schwab S, Horn M, Spranger M, De Georgia M, von Kummer R. ‘Malignant’ middle cerebral artery territory infarction: clinical course and prognostic signs. *Arch Neurol.* 1996;53:309–315.
16. Brott T, Marler JR, Olinger CP, et al. Measurements of acute cerebral infarction: lesion size by computed tomography. *Stroke.* 1989;20:871–875.
17. Harraf F, Sharma AK, Brown MM, Lees KR, Vass RI, Kalra L. A multicentre observational study of presentation and early assessment of acute stroke. *BMJ.* 2002;325:17–20.
18. Shih WJ, Domstad PA, DeLand FH. Opportunistic intracranial infection in AIDS detection by technetium- ^{99m}Tc DTPA brain scintigraphy. *J Nucl Med.* 1986;27:498–501.
19. Siegel BA, Meidinger R, Elliott AJ, et al. Experimental cerebral microembolism: multiple tracer assessment of brain edema. *Arch Neurol.* 1972;26:73–77.
20. Lansberg MG, Albers GW, Beaulieu C, Marks MP. Comparison of diffusion-weighted MRI and CT in acute stroke. *Neurology.* 2000;54:1557–1561.
21. Bryan RN, Levy LM, Whitlow WD, Killian JM, Preziosi TJ, Rosario JA. Diagnosis of acute cerebral infarction: comparison of CT and MR imaging. *AJNR.* 1991;12:611–620.
22. Perkins CJ, Kahya E, Roque CT, Roche PE, Newman GC. Fluid-attenuated inversion recovery and diffusion- and perfusion-weighted MRI abnormalities in 117 consecutive patients with stroke symptoms. *Stroke.* 2001;32:2774–2781.

23. Hossmann KA, Hoehn-Berlage M. Diffusion and perfusion MR imaging of cerebral ischemia. *Cerebrovasc Brain Metab Rev*. 1995;7:187–217.
24. Fiehler J, Foth M, Kucinski T, et al. Severe ADC decreases do not predict irreversible tissue damage in humans. *Stroke*. 2002;33:79–86.
25. Ringer TM, Neumann-Haefelin T, Sobel RA, Moseley ME, Yenari M. Reversal of early diffusion-weighted magnetic resonance imaging abnormalities does not necessarily reflect tissue salvage in experimental cerebral ischemia. *Stroke*. 2001;32:2362–2369.
26. Marchal G, Serrati C, Rioux P, et al. PET imaging of cerebral perfusion and oxygen consumption in acute ischaemic stroke: relation to outcome. *Lancet*. 1993;341:925–927.
27. Shimosegawa E, Hatazawa J, Inugami A, et al. Cerebral infarction within six hours of onset: prediction of completed infarction with technetium-99m-HMPAO SPECT. *J Nucl Med*. 1994;35:1097–1103.
28. Slosman DO, Ludwig C, Zerarka S, et al. Brain energy metabolism in Alzheimer's disease: ^{99m}Tc-HMPAO SPECT imaging during verbal fluency and role of astrocytes in the cellular mechanism of ^{99m}Tc-HMPAO retention. *Brain Res Brain Res Rev*. 2001;36:230–240.

