Clinical Chemistry 49:4
562–569 (2003)

Prognostic Use of Circulating Plasma Nucleic Acid Concentrations in Patients with Acute Stroke

Timothy H. Rainer,[1] Lawrence K.S. Wong,[2] Wynnie Lam,[3] Eddie Yuen,[1]
Nicole Y.L. Lam,[1] Constantine Metreweli,[3] and Y.M. Dennis Lo[4*]

Background: **At present there is no simple, accurate blood test that may be used to determine the severity of stroke or to predict mortality and morbidity in stroke patients presenting to emergency departments.**
Methods: **Patients with stroke-like symptoms who presented to an emergency department of a university hospital in Hong Kong were recruited for the study. DNA extracted from patients' plasma was analyzed for the *β-globin* gene with a fluorescent-based PCR test. The primary outcome measures were in-hospital and 6-month mortality and morbidity using the post-stroke modified Rankin Score.**
Results: **Among the 88 consecutive patients recruited to the study, 70 (80%) had ischemic stroke, 11 (13%) had intracerebral hemorrhage, and 7 (8%) had transient ischemic attacks. Median plasma DNA concentrations taken within 3 h of symptom onset were higher in patients who died compared with those who survived at discharge (6205 vs 1334 kilogenome-equivalents/L; $P = 0.03$). Among patients with NIH Stroke Scale scores >8, median plasma DNA concentrations were higher in patients who died compared with those who survived to 6 months (2273 vs 968 kilogenome-equivalents/L; $P = 0.002$). Plasma DNA concentrations correlated with the volume of cerebral hematoma ($r = 0.66$; $P = 0.03$). Plasma DNA concentrations >1400 kilogenome-equivalents/L had a sensitivity of 100% and a specificity of 74.4% for predicting hospital mortality after stroke, and the area under the ROC curve was 0.89 (95% confidence interval, 0.80–0.94). The adjusted odds ratio for plasma DNA concentrations predicting 6-month mortality was 1.6 (1.1–2.4; $P = 0.03$) and for predicting 6-month post-Rankin Score >2 was 1.8 (1.0–3.3; $P = 0.05$).**
Conclusion: **Plasma DNA concentrations correlate with stroke severity and may be used to predict mortality and morbidity in the emergency room.**

Stroke ranked as the second leading cause of all deaths worldwide in 1990, accounting for 4.4 million victims *(1)*, and is also currently the leading cause of brain injury in adults *(2)*. Preventive strategies have led to a decrease in the rate of stroke attack and death, but these improvements have been offset in part by the growth of an aging population such that by the year 2020, stroke is likely to maintain its ranking as the second leading cause of global mortality *(3, 4)*.

Unlike acute coronary syndrome, which has many nonspecific and specific plasma or serum markers that may be used to both diagnose and assess the severity of myocardial infarction, no similar established markers exist for patients with stroke. The blood–brain barrier is compromised in many patients with stroke, and the liberation of neurobiochemical protein markers into the circulation may allow the pathophysiology, progress, and prognosis of patients with cerebrovascular disease to be further evaluated *(5–8)*. Although increased concentrations of several neurobiochemical protein markers have been detected in the peripheral blood of patients with stroke, to date none has found a place in clinical practice. Circulating DNA in plasma is altered both qualitatively and quantitatively in a variety of conditions, including pregnancy *(9–11)*, cancer *(12, 13)*, graft rejection *(14)*, and trauma *(15)*. Although the mechanisms by which nucleic acids are released into the circulation are unknown, it is likely that cell death is one major factor *(16, 17)*. As both hemorrhagic and ischemic stroke *(18)* involve cell death and disruption of the blood–brain barrier, we hypothesized that DNA would be liberated into the plasma early after the onset of stroke and might be useful for assessing disease severity and for predicting mortality.

[1] Accident and Emergency Medicine Academic Unit and Departments of [2] Medicine and Therapeutics, [3] Diagnostic Radiology and Organ Imaging, and [4] Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong Special Administrative Region.
*Address correspondence to this author at: Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Room 38023, 1/F Clinical Sciences Bldg., 30-32 Ngan Shing St., Shatin, New Territories, Hong Kong Special Administrative Region. E-mail loym@cuhk.edu.hk.
Received September 5, 2002; accepted January 24, 2003.

Materials and Methods

STUDY DESIGN AND PATIENTS

Approval was obtained from the Institutional Review Board of the Chinese University of Hong Kong to conduct a prospective study investigating the role of plasma DNA in the diagnosis and prognosis of patients presenting with stroke-like syndromes at the Prince of Wales Hospital. The Prince of Wales Hospital is a 1400-bed university hospital, based in the New Territories of Hong Kong, that serves a population of ~1 500 000.

Eligible patients ≥18 years of age presenting to the emergency department with a stroke-like syndrome were recruited consecutively into the study. Exclusion criteria included multiple trauma, craniocerebral or cervical trauma, meningitis, encephalitis or other sepsis, hypertensive encephalopathy, intracranial tumor, seizures with persistent neurologic signs (Todd paralysis), Bell palsy, migraine, metabolic disturbances (e.g., hypo- and hyperglycemia), post-cardiac arrest, drug overdose, endocrine disorders (e.g., myxedema), renal failure, psychiatric syndromes, or shock with hypoperfusion. Patients were also excluded if the time from symptom onset to blood sampling was >24 h. Informed, written consent was obtained either from the patient or a relative in all cases. Twenty-four healthy age- and sex-matched individuals were recruited as controls.

Four groups of operators who undertook the responsibilities of clinical data collection, final clinical outcome determination, neuroimaging interpretation, and analysis of DNA assays were blinded to one other.

DATA COLLECTION, DEFINITIONS, AND DIAGNOSTIC IMAGING

Stroke was defined as the acute occurrence of focal neurologic signs lasting for more than 24 h in a different neuroanatomical location from that of any previous stroke, or worsening of an existing deficit that lasted for more than 1 week or more than 24 h if accompanied by a new lesion on neuroimaging *(19)*. Stroke-like syndrome was defined according to the following criteria: (*a*) the patient had a facial droop, arm drift or weakness, or abnormal speech (compatible with the Cincinnati Prehospital Stroke Scale) *(20)*; or (*b*) the patient had an altered level of consciousness with no obvious associated seizures, hyperglycemia, or hypoglycemia (compatible with the Los Angeles Prehospital Stroke Scale *(21, 22)*.

Demographic and previous medical data were collected, including age, sex, symptom onset time, history of previous strokes, seizures, hypertension, diabetes mellitus, ischemic heart disease, atrial fibrillation, hyperlipidemia, smoking, and antithrombotic and other medication. Each patient's previous health was assessed with use of the pre-stroke modified Rankin Scale, a simplified overall assessment of function in which a score of 0 indicates the absence of symptoms and a score of 5 indicates severe disability *(23)*.

To determine the exact nature and cause of the stroke-like syndrome, patients received a standard clinical, laboratory, and imaging workup, including cerebral computed axial tomography (CT)[5] without contrast enhancement *(24, 25)* and magnetic resonance imaging (MRI), including a diffusion-weighted MRI study *(26–28)*. All CT scans were performed with a gradient echo HiSpeed Advantage Unit. Axial 5/5-mm scans were done for the posterior cranial fossa, and 10/10-mm scans were then performed up to the vortex-mix space. MRI scans were performed with a 1.5-Tesla scanner (Sonata; Siemens). The sequences included spin echo, T1 axial [repetition time (TR), 425 ms; time to echo (TE), 14 ms; number of excitation, 2; slice thickness, 5 mm; slice gap, 0.5 mm; 192 × 256 matrix]; turbo spin echo, T2 axial (TR, 2500 ms; TE, 120 ms; number of excitation, 1; slice thickness, 5 mm; slice gap, 0.5 mm; 192 × 256 matrix); and single-shot echo planar imaging diffusion-weighted axial images (TR, 180 ms; TE, 122 ms; slice thickness, 5 mm; matrix, 128 × 128; echo planar imaging factor of 90). Three different diffusion gradients were used, giving *b* values of 0, 500, and 1000 s/mm^2.

The volume of infarct was measured on both CT films and MRI diffusion scans, whereas the volume of an acute hematoma was measured on CT films and T1-weighted MRI scans. CT (n = 88) and MRI (n = 71) scans were not possible for all cases because some patients were too ill for two scans and some patients had contraindications for MRI.

Patients were classified as having a transient ischemic attack if their symptoms and signs resolved within 24 h. Stroke cases were classified into one of two groups: intracerebral hemorrhage or cerebral infarction. Nonhemorrhagic cases were further classified according to definitions used in the Trial of Org 10172 in Acute Stroke Treatment (TOAST) trial *(29)*.

The severity of stroke was assessed clinically using two methods. The National Institutes of Health Stroke Scale (NIHSS) is a 42-point scale that quantifies neurologic deficit in 11 categories such that normal function with no deficit receives a score of 0 *(30)*. The Glasgow Coma Score is a 13-point score that ranges from 3 to 15 and assesses visual, motor, and verbal responses to stimulus such that a normal response receives a score of 15 and 3 indicates severe dysfunction *(31)*.

PREPARATION OF PLASMA DNA AND REAL-TIME PCR

A 10-mL blood sample was withdrawn from the antecubital vein of each patient, collected into tubes containing EDTA, and centrifuged at 1500*g* for 5 min; plasma was then transferred into plain polypropylene tubes and stored at −80 °C pending further processing.

DNA was extracted from 200-μL plasma samples with

[5] Nonstandard abbreviations: CT, computerized tomography; MRI, magnetic resonance imaging; TR, repetition time; TE, time to echo; NIHSS, National Institutes of Health Stroke Scale; and CI, confidence, interval.

use of a QIAamp Blood Kit (Qiagen) according to the "blood and body fluid protocol" as recommended by the manufacturer *(12)*. The theoretical and practical aspects of real-time quantitative PCR have been described in detail elsewhere, and the whole process takes ~3 h *(11, 32–34)*.

Plasma DNA was measured by a real-time quantitative PCR assay for the *β-globin* gene, which is present in all nucleated cells of the body *(11)*. The *β-globin* PCR system consists of the amplification primers beta-globin-354F (5′-GTG CAC CTG ACT CCT GAG GAG A-3′) and beta-globin-455R (5′-CCT TGA TAC CAA CCT GCC CAG-3′) and a dual-labeled fluorescent PCR probe *beta-globin*-402T [5′-(VIC®)AAG GTG AAC GTG GAT GAA GTT GGT GG(TAMRA)-3′ *(11)*, where TAMRA is 6-carboxytetramethylrhodamine]. The PCR probe contained a 3′-blocking phosphate group to prevent probe extension during PCR.

When applied to serial dilutions of human genomic DNA, this real-time quantitative *β-globin* PCR assay was able to detect the DNA equivalent from a single cell. The imprecision of this system has been reported previously, with a CV of the threshold cycle of 1.1% *(11)*. The expression of quantitative results as kilogenome-equivalents/L was as described previously *(11)*. One genome-equivalent was defined as the amount of a particular target sequence contained in a single diploid human cell.

OUTCOME

The primary outcome measures were mortality and post-stroke modified Rankin Score at 6 months after the onset of symptoms. Other outcome measures included mortality in hospital or within 28 days, whichever was sooner, and loss of quality of life, defined as the 6-month post-stroke modified Rankin Score minus the pre-stroke modified Rankin Score.

STATISTICAL ANALYSIS

Descriptive statistics and data comparison tests (χ^2, Fisher exact, Mann–Whitney, and Kruskal–Wallis tests) were carried out using Statview® for Windows, Ver. 5.0, Statistical Analysis Software (Abacus Concepts, SAS Institute) as appropriate. Correlations were determined using Spearman rank or Kruskal–Wallis tests, whereas ROC curve analysis was carried out using the MedCalc 5.0 software. Multiple logistic regression analysis using backward stepwise selection procedures to exclude those variables with P values >0.05 was built, and P values were calculated using the Wald test. The Hosmer and Lemeshow test was used to confirm the goodness of fit.

Results

BASELINE CHARACTERISTICS

The characteristics of 88 adult patients who were enrolled in the study with a stroke-like syndrome are shown in Table 1. Although the majority of patients were elderly and had several risk factors for stroke, 71 (81%) had no

Table 1. Characteristics of the 88 patients presenting to hospital with stroke.[a]

Characteristics	Value
Age, years	
Median (interquartile range)	74 (16)
Range	50–92
Male sex, no. of patients (%)	45 (51)
Stroke risk factors, no. of patients (%)	
Hypertension	53 (60)
Diabetes mellitus	21 (24)
Ischemic heart disease	11 (13)
Atrial fibrillation	15 (17)
Hyperlipidemia	9 (10)
Active smoker	18 (21)
Ex-smoker	22 (25)
Previous stroke	24 (27)
Pre-stroke modified Rankin Score, no. of patients (%)	
Asymptomatic	71 (81)
No significant disability	14 (16)
Slight disability	2 (2)
Moderate disability	0
Moderately severe disability	1 (1)
Severe disability	0
Time from onset of symptoms to blood sample, h	
Median (interquartile range)	8 (12)
Range	1–24
Pulse rate, beats/min	
Median (interquartile range)	77 (22)
Range	24–172
Blood pressure, mmHg	
Systolic	
Median (interquartile range)	169 (55)
Range	100–272
Diastolic	
Median (interquartile range)	88 (26)
Range	40–142
Blood glucose, mmol/L	
Median (interquartile range)	6.8 (3.5)
Range	4.2–20
Glasgow Coma Score, no. of patients (%)	
3–8	4 (5)
9–12	9 (10)
13–15	75 (85)
NIHSS score, no. of patients (%)	
0–1	7 (8)
2–8	40 (46)
9–40	41 (47)
Stroke types, no. of patients (%)	
Infarct	70 (80)
Hemorrhage	11 (13)
Transient ischemic attack, no. of patients (%)	7 (8)
TOAST,[b] no. of patients (%)	
Cardioembolism	8 (9)
Hemorrhage	11 (13)
Large artery atherosclerosis	29 (33)
Small vessel occlusion or lacunae	3 (3)
Undetermined	37 (42)

[a] All continuous variables are expressed as medians (interquartile range) and range. Numbers may not sum to 100 because of rounding, multiple factors (e.g., risk factors), or absent data.

[b] TOAST, Trial of Org 10172 in Acute Stroke Treatment *(29)*.

significant health disability before their acute admission, as assessed using the pre-stroke modified Rankin Score. Six patients died within 28 days of hospital admission and 11 within 6 months. In 11 cases, there were no signs of hemorrhage or infarction on neuroimaging, but persistent clinical features of stroke were present that were unexplained by any other cause. These cases were classified as having had a cerebral infarction. The majority of patients presented within the first few hours of symptom onset. The 24 individuals (mean age, 70 years; 11 males) in the healthy control group did not differ significantly from the patient group in either age ($P = 0.23$) or sex ($P = 0.64$).

UNIVARIATE ANALYSIS OF PLASMA DNA AND OTHER VARIABLES WITH OUTCOME

In the univariate analysis, three variables significantly differentiated patients who died from those who survived at 6 months after presentation: plasma DNA, NIHSS score, and Glasgow Coma Score (Table 2). Median plasma DNA concentrations taken within the first 3 h of symp-

Table 2. Comparison of factors for predicting 6-month mortality and post-stoke modified Rankin Score in 88 patients with stroke.[a]

	Mortality			Post-stroke modified score		
Factor	Survived (n = 77)	Died (n = 11)	P^b	≤2 (n = 48)	>2 (n = 40)	P^b
Age, years						
Median (interquartile range)	73 (16)	75 (11.5)	0.16	72 (17)	76 (14)	0.13
Minimum–maximum	50–92	55–90		50–91	54–92	
Male sex, no. of patients (%)	38 (49)	7 (64)	0.38	24 (50)	24 (60)	0.82
Time from symptom onset to blood sampling, h						
Median (interquartile range)	8.0 (12.0)	6.5 (13.0)	0.60	10.0 (13.0)	7.0 (9.0)	0.21
Minimum–maximum	0.5–24	0.5–21		1.5–22.0	0–24	
Pre-stroke modified Rankin Scale, no. of patients (%)						
0–1	74 (96)	11 (100)	1.0	47 (98)	38 (95)	0.59
2–4	3 (4)			1 (2)	2 (5)	
Glasgow Coma Scale						
Median (interquartile range)	15 (0)	11 (7.5)	0.004	15 (0)	15 (3.5)	0.008
Minimum–maximum	14–15	6–15		10–15	4–15	
NIHSS score, no. of patients (%)						
0–1	7 (9)		0.37	5 (10)	2 (5)	0.007
2–8	36 (47)	4 (36)		28 (58)	12 (30)	
>8	34 (44)	7 (64)		15 (31)	26 (65)	
Systolic blood pressure, mmHg						
Median (interquartile range)	170 (57)	160 (49)	0.86	171 (52)	160 (59)	0.72
Minimum–maximum	100–263	114–272		102–263	100–272	
Diastolic blood pressure, mmHg						
Median (interquartile range)	87 (28)	94 (23)	0.12	88 (23)	91 (32)	0.49
Minimum–maximum	40–142	70–134		40–136	53–142	
Risk factors, no. of patients (%)						
Hypertension	46 (60)	7 (64)	1.0	28 (58)	25 (63)	0.69
Diabetes mellitus	20 (26)	1 (9)	0.45	11 (23)	10 (25)	0.82
Ischemic heart disease	8 (10)	3 (27)	1.0	5 (10)	6 (15)	0.52
Atrial fibrillation	11 (14)	4 (36)	1.0	6 (13)	9 (23)	0.21
Previous stroke	19 (25)	4 (36)	1.0	13 (27)	10 (25)	0.82
Lesion volume on MRI, cm^3						
Median (interquartile range)	1.7 (15.1)	5.5 (55.4)	0.36	1.1 (8.8)	5.6 (43.6)	0.05
Minimum–maximum	0–220.7	0.8–105.1		0–193.3	0–220.7	
Lesion volume on CT, cm^3						
Median (interquartile range)	0.1 (3.7)	70.0 (234.8)	0.13	0.1 (3.1)	0.6 (50.0)	0.16
Minimum–maximum	0–225.0	0–518.5		0–151.2	0–518.5	
Plasma DNA, $\times 10^3$ kilogenome-equivalents/L						
Median (interquartile range)	1.0 (0.6)	2.0 (1.9)	0.0003	1.0 (0.5)	1.3 (1.3)	0.02
Minimum–maximum	0.3–7.1	0.9–8.3		0.3–5.2	0.3–8.3	

[a] Continuous variables are given as medians (interquartile range) and minimum–maximum range. Categorical variables are given as values (percentages).
[b] *P* values were derived using the Mann–Whitney test, χ^2 test, or Fisher exact test as appropriate.

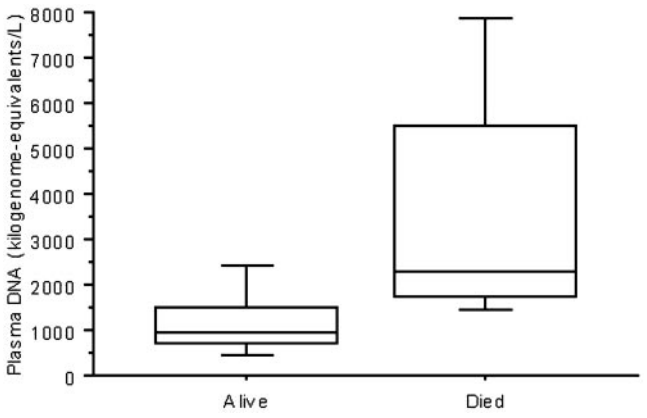

Fig. 1. Plasma DNA concentrations and 6-month mortality in patients presenting with stroke.

Plasma DNA concentrations as determined by real-time quantitative PCR for the *β-globin* gene (*y axis*) in patients who survived or died within 6 months of presentation (*x axis*). The *lines inside* the *boxes* denote medians; the *boxes* indicate the interval between the 25th and 75th percentiles. The *whiskers* denote the interval between the 10th and 90th percentiles. The difference between the groups is statistically significant ($P = 0.002$, Mann–Whitney test).

toms were fivefold higher in patients who died than in those who survived (6205 vs 1334 kilogenome-equivalents/L; $P = 0.03$), whereas the highest single result of 8272 kilogenome-equivalents/L occurred in a patient who later died.

In those patients who presented with a NIHSS score >8, median plasma DNA concentrations were higher in those who died compared with those patients who survived to 6 months (2273 vs 968 kilogenome-equivalents/L; $P = 0.002$, Fig. 1).

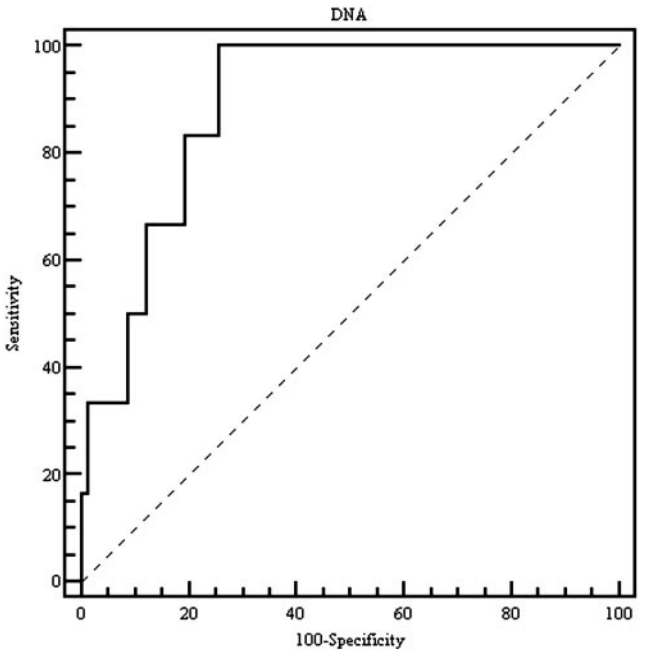

Fig. 2. ROC curve analysis of plasma DNA concentrations for prediction of mortality within 28 days of onset of symptoms.

Values indicated on the *x* and *y axes* are expressed in percentages. Seven patients died. The area under the curve is 0.89 (95% CI, 0.80–0.94).

The ROC curve analysis comparing the sensitivity and specificity of plasma DNA concentration in the six patients who died within 28 days of hospital admission is shown in Fig. 2. The area under the curve was 0.89 [95% confidence interval (CI), 0.80–0.94]. At a plasma DNA cutoff of 1400 kilogenome-equivalents/L, the optimal sensitivity and specificity were 100% (95% CI, 100–100%) and 74.4% (63.6–83.4%), respectively. At this cutoff, the odds ratio was 3.9, and the negative predictive value was 100%.

Median plasma DNA concentrations were 30% higher in patients with a 6-month modified post-Rankin Score >2 compared with patients who scored ≤2 (Table 2). In the univariate analysis, NIHSS score, MRI lesion volume, and Glasgow Coma Score also differentiated these two groups of patients.

Plasma DNA concentrations correlated with the Glasgow Coma Score ($H = 12.127$; $P = 0.002$, Kruskal–Wallis test), cerebral hemorrhage volume ($r = 0.66$; $P = 0.03$, Spearman rank), post-stroke modified Rankin Score ($H = 21.808$; $P = 0.001$, Kruskal–Wallis), and quality of life loss ($H = 20.696$; $P = 0.004$, Kruskal–Wallis).

Logistic regression model for predicting 6-month mortality and modified post-Rankin score >2

All variables from Table 2 were initially entered into the logistic regression model, and the most insignificant variables were removed from the model in stepwise fashion. Table 3 shows the adjusted odds ratios of independent variables for predicting 6-month mortality and a post-stroke modified Rankin Score >2. Lesion volume as measured by CT and plasma DNA concentrations were the only significant variables for predicting 6-month mortality. NIHSS groups >8 and plasma DNA concentrations were the only significant variables for predicting a 6-month modified post-stroke modified Rankin Score >2.

For every 460 kilogenome-equivalents/L increase in plasma DNA, the associated increased risk of death within 6 months of stroke was 58.5% (95% CI, 5–239%), whereas for every 1.3-cm^3 increase in lesion volume as determined by CT, the increased risk of death within 6 months of stroke was 14% (3.2–125%). For every 600 kilogenome-equivalents/L increase in plasma DNA, the associated increased risk of a 6-month post-stroke modified Rankin Score >2 was 82% (0–331%).

Discussion

This is the first study to show that circulating plasma DNA concentrations, assessed by measuring *β-globin* gene concentrations by real-time PCR, increase in patients in the first 24 h of acute stroke. We have also shown that plasma DNA measurements may be useful for early risk stratification and for predicting in-hospital and 6-month disability and mortality. The greatest differences in

Table 3. Logistic regression model of factors for predicting 6-month mortality and post-stroke modified Rankin Score >2 after stroke (n = 88).[a]

Factor	Adjusted odds ratio for mortality (95% CI)	*P*	Adjusted odds ratio for post-stroke modified Rankin Score >2 (95% CI)	*P*
Lesion volume on CT, × 10 cm^3	1.14 (1.03–1.25)	<0.01		
Plasma DNA, × 10^3 kilogenome-equivalents/L	1.58 (1.05–2.39)	0.03	1.82 (1.00–3.31)	0.05
NIHSS group >8			3.42 (1.36–8.59)	<0.01

[a] Logistic regression model using backward stepwise selection procedures was built using variables from Table 2. Only lesion volume as determined by CT scanning and plasma DNA concentrations were significantly associated with 6-month mortality, whereas only NIHSS group >8 and plasma DNA concentrations were significantly associated with a post-stroke modified Rankin Score >2. The Hosmer and Lemeshow goodness-of-fit test was used to confirm that the models fitted the data: mortality, χ^2 test = 5.432; $P = 0.71$; post-stroke modified Rankin Score, χ^2 test = 5.402; $P = 0.71$.

plasma DNA concentrations between patients with good and poor outcomes occurred within 3 h of the onset of symptoms. Plasma DNA concentrations correlated with stroke severity and may also be useful for risk stratification and for predicting the 6-month modified post-Rankin outcome scores in patients with clinical stroke but negative neuroimaging. A cutoff value of ≥1400 kilogenome-equivalents/L yielded a sensitivity of 100%, a specificity of 74.4%, an odds ratio of 3.9, and a negative predictive value of 100% for determining mortality.

Previous studies have reported increased plasma or serum neurobiochemical markers, e.g., S-100 protein, neuron-specific enolase, and *N*-acetylaspartate, in the peripheral circulation of patients after stroke but failed to demonstrate that these markers offered any additional advantage over current clinical assessment scales, risk factors, or neuroradiologic techniques *(5–8)*. Correlations have been observed between marker values, infarct size, and NIHSS scores, and increased concentrations predict disability and mortality. However, in our study we demonstrated not only that plasma DNA concentrations are increased in patients with stroke and correlate with stroke severity, but also that these concentrations considerably improve our ability to objectively predict mortality and the 6-month post-stroke modified Rankin Score over and above what is offered by current neuroimaging techniques and subjective clinical scoring methods. Plasma DNA does not appear to help differentiate between hemorrhagic stroke and cerebral infarction; thus, any role in guiding therapeutic intervention is currently unclear.

The mechanisms by which circulating cell-free DNA increases after stroke require further study but are likely to be a result of increased liberation from damaged cells. Stroke involves a complicated cascade of events involving cerebral ischemia, altered cerebral blood flow, inflammation, the production of reactive oxygen radicals, neuronal necrosis and apoptosis, and neurologic dysfunction *(35–39)*. DNA may be liberated from cells undergoing apoptosis or necrosis, and increased concentrations have been noted in other conditions involving organ, tissue, and cellular injury, such as cancer *(12, 13)* and major trauma *(15)*. Which of these mechanisms are involved in patients with stroke is not known, but cellular ischemia and tissue infarction undoubtedly occur and are associated with disruption of the blood–brain barrier such that increased local liberation of DNA from cells might produce increased systemic plasma concentrations. Although the precise mechanisms of clearance are unknown, it is known that human plasma DNA has an extremely short half-life in the circulation *(40)*, and the rapid kinetics suggest that it may be useful for monitoring disease progress in acute illness involving tissue injury. Because β-globin is found in all nuclear cells in the body, we cannot rule out the possibility that other non-cerebral tissue pathologies associated with cell death or DNA release may contribute to increased plasma DNA. Patients with stroke are frequently elderly and are likely to have other preexisting diseases.

Significant positive correlations were observed between plasma DNA concentrations, lesion size, and the post-stroke modified Rankin Score. Multiple factors, time relationships, and small cerebral abnormalities at critical sites may dramatically affect the clinical presentation and outcome, but in general it appears that patients with more dramatic clinical presentations and outcomes had a general trend toward increased plasma DNA concentrations. Plasma DNA concentrations correlated with the hemorrhagic volume, as measured by CT, but not with lesion size as measured by MRI (data not shown). Infarct size measured by MRI was calculated based on diffusion-weighted images, which in turn reflect the area of the brain with abnormal water diffusion. MRI therefore shows areas that may be ischemic but not infarcting; consequently, correlations with a marker of cell death, i.e., plasma DNA, may not be evident.

Further studies are required to validate and refine the optimal plasma DNA cutoff values for diagnosis and prognosis, and future work should address relationships with time, plasma DNA kinetics, and morbidity outcomes. Studies on serial samples may allow us to investigate some of the pathologic changes that underlie the progression of stroke, optimize the sampling times, and monitor the effects of treatment.

Technologic advances may further enhance the usefulness of plasma DNA measurement in acute medicine. For example, at present the use of a column-based DNA extraction method and real-time PCR analysis allows plasma DNA results to be available within 3 h of sam-

pling. The recent developments of rapid capillary-based instrumentation for quantitative PCR analysis may allow this time to be reduced further, to 90 min *(41)*.

In conclusion, this study is the first to report a DNA-based marker for stroke, and the findings suggest that measuring plasma DNA may be a potentially useful, relatively quick, and noninvasive test for monitoring patients presenting with stroke-like symptoms. A simple DNA test might be used to predict mortality and morbidity and to stratify patients entering into clinical trials. Where CT and/or MRI are either unavailable or yield no obvious acute abnormalities, a plasma DNA test may also help to predict likely mortality or functional outcome.

This project was funded by the Research Grants Council (Direct Grant Allocation 2040804). Although no commercial sponsorship was available to conduct and complete this study, a patent application describing the technology reported here has been filed and is owned by The Chinese University of Hong Kong. An appendix containing additional information is available with the online version of this article at http://www.clinchem.org/content/vol49/issue4/.

References

1. Murray CJL, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. Lancet 1997;349:1498–505.
2. Broderick JP, Brott T, Tomsick T, Huster G, Miller R. The risk of subarachnoid and intracerebral hemorrhages in blacks as compared with whites. N Engl J Med 1992;326:733–6.
3. Thorvaldsen P, Kuulasmaa K, Rajakangas AM, Rastenyte D, Sarti C, Wilhelmsen L. Stroke trends in the WHO MONICA project. Stroke 1997;28:500–6.
4. Thorvaldsen P, Davidsen M, Bronnum-Hansen H, Schroll M. Stable stroke occurrence despite incidence reduction in an aging population: stroke trends in the Danish monitoring trends and determinants in cardiovascular disease (MONICA) population. Stroke 1999;30:2529–34.
5. Abraha HD, Butterworth RJ, Bath PM, Wassif WS, Garthwaite J, Sherwood RA. Serum S-100 protein, relationship to clinical outcome in acute stroke. Ann Clin Biochem 1997;34:366–70.
6. Buttner T, Weyers S, Postert T, Sprengelmeyer R, Kuhn W. S-100 protein: serum marker of focal brain damage after ischemic territorial MCA infarction. Stroke 1997;28:1961–5.
7. Stevens H, Jakobs C, de Jager AE, Cunningham RT, Korf J. Neuron-specific enolase and N-acetyl-aspartate as potential peripheral markers of ischemic stroke. Eur J Clin Invest 1999;29:6–11.
8. Wunderlich MT, Ebert AD, Kratz T, Goertler M, Jost S, Herrmann M. Early neurobehavioural outcome after stroke is related to release of neurobiochemical markers of brain damage. Stroke 1999;30:1190–5.
9. Lo YMD, Hjelm NM, Fidler C, Sargent IL, Murphy MF, Chamberlain PF, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N Engl J Med 1998;339:1734–8.
10. Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CWG. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485–7.
11. Lo YMD, Tein MSC, Lau TK, Haines CJ, Leung TN, Poon PM, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet 1998;62:768–75.
12. Chen XQ, Stroun M, Magnenat JL, Nicod LP, Kurt AM, Lyautey J, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 1996;2:1033–5.
13. Nawroz H, Koch W, Anker P, Stroun M, Sidransky D. Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med 1996;2:1035–7.
14. Lo YMD, Tein MSC, Pang CCP, Yeung CK, Tong KL, Hjelm NM. Presence of donor-specific DNA in plasma of kidney and liver-transplant recipients. Lancet 1998;351:1329–30.
15. Lo YMD, Rainer TH, Chan LYS, Hjelm NM, Cocks RA. Plasma DNA as a prognostic marker in trauma patients. Clin Chem 2000;46:319–23.
16. Fournie GJ, Martres F, Pourrat JP, Alary C, Rumeau M. Plasma DNA as cell death marker in elderly patients. Gerontology 1993;39:215–21.
17. Fournie GJ, Courtin JP, Laval F, Chale JJ, Pourrat JP, Pujazon MC, et al. Plasma DNA as a marker of cancerous cell death. Investigations in patients suffering from lung cancer and in nude mice bearing human tumours. Cancer Lett 1995;91:221–7.
18. Williams GR, Jiang JG, Matchar DB, Samsa GP. Incidence and occurrence of total (first-ever and recurrent) stroke. Stroke 1999;30:2523–8.
19. Special report from the National Institute of Neurological Disorders and Stroke: classification of cerebrovascular diseases III. Stroke 1990;21:637–76.
20. Kothari RU, Pancioli A, Liu T, Brott T, Broderick J. Cincinnati Prehospital Stroke Scale: reproducibility and validity. Ann Emerg Med 1999;33:373–8.
21. Kidwell CS, Saver JL, Schubert GB, Eckstein M, Starkman S. Design and retrospective analysis of the Los Angeles Prehospital Stroke Screen (LAPSS). Prehosp Emerg Care 1998;2:267–73.
22. Kidwell CS, Starkman S, Eckstein M, Weems K, Saver JL. Identifying stroke in the field: prospective validation of the Los Angeles Prehospital Stroke Screen (LAPSS). Stroke 2000;31:71–6.
23. van Sweiten JC, Koudstaal PJ, Visser MC, Shouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19:604–7.
24. Gilman S. Imaging the brain. N Engl J Med 1998;338:889–96.
25. Davis KR, Acerman RH, Kistler JP. Computed tomography of cerebral infarction: hemorrhagic, contrast enhancement, and time of appearance. Comput Tomogr 1977;1:71–86.
26. Linfante I, Llinas RH, Caplan LR. MRI features of intracerebral hemorrhage within 2 hours from symptom onset. Stroke 1999;30:2263–7.
27. Schellinger PD, Jansen O, Fiebach JB. A standardised MRI stroke protocol: comparison with CT in hyperacute intracerebral hemorrhage. Stroke 1999;30:765–8.
28. Tong DC, Yenari MA, Albers GW. Correlation of perfusion and diffusion weighted MRI with NIHSS score in acute (<6.5 hour) ischemic stroke. Neurology 1998;50:864–70.
29. Adams HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke: definitions for use in a multicenter clinical trial: TOAST: Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24:35–41.
30. Spiker J, Kongable G. The NIH Stroke Scale: its importance and practical application in the clinical setting. Stroke Intervent 2000;2:7–14.
31. Teasdale G, Jennet B. Assessment of coma and impaired consciousness: a practical scale. Lancet 1974;2:81–4.
32. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res 1996;6:986–94.
33. Luthra R, McBride JA, Cabanillas F, Sarris A. Novel 5′ exonucle-

ase-based real-time PCR assay for the detection of t(14;18)(q32; q21) in patients with follicular lymphoma. Am J Pathol 1998;153: 63–8.

34. Holland PM, Abramson RD, Watson R, Gelfand DH. Detection of specific polymerase chain reaction product by utilizing the 5′-3′ exonuclease activity of Thermus aquaticus DNA polymerase. Proc Natl Acad Sci U S A 1991;88:7276–80.

35. Yang GY, Pang L, Ge HL, Tan M, Ye W, Liu XH, et al. Attenuation of ischemia-induced mouse brain injury by SAG, a redox-inducible antioxidant protein. J Cereb Blood Flow Metab 2001; 21:722–33.

36. Sairanen T, Carpen O, Karjalainen-Lindsberg ML, Paetau A, Turpeinen U, Kaste M, et al. Evolution of cerebral tumor necrosis factor-α production during human ischemic stroke. Stroke 2001; 32:1750–8.

37. Graham SH, Chen J. Programmed cell death in cerebral ischemia. J Cereb Blood Flow Metab 2001;21:99–109.

38. Reed JC. Mechanisms of apoptosis. Am J Pathol 2000;157: 1415–30.

39. MacManus JP, Buchan AM. Apoptosis after experimental stroke: fact or fashion? J Neurotrauma 2000;17:899–914.

40. Lo YMD, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. Am J Hum Genet 1999;64:218–24.

41. Wittwer CT, Ririe KM, Andrew RV, David DA, Gundry RA, Balis UJ. The LightCycler: a microvolume multisample fluorimeter with rapid temperature control. Biotechniques 1997;22:176–81.