

Letters to the Editor

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Prevalence of Stroke and Stroke-Related Disability

To the Editor:

Bonita and colleagues¹ provide valuable information on the prevalence of stroke and the proportion with persisting sequelae. Their conclusions, drawn on the basis on an actuarial model, agree well with the results from a population-based survey from Nord-Trøndelag County, Norway.² This study comprised 74 977 persons, including permanent nursing home residents; the attendance rate was 88.1%. The raw prevalence rate of stroke was 1850 per 100 000 in the population aged ≥ 20 years and 960 per 100 000 when standardized to the entire European population. However, when the sensitivity and specificity of the screening question³ are taken into account, one may have to adjust the prevalence estimate downward to approximately 1100 per 100 000.⁴ In the Table, age-specific prevalence estimates from the Nord-Trøndelag and the Auckland studies are compared.

The Nord-Trøndelag study² provides figures for self-reported motor impairments, whereas the Auckland study¹ reports the prevalence rate of patients with self-reported incomplete recovery and those who need help in activities of daily living. The results of the two studies (for both sexes combined) are summarized in the Table. Despite focusing on different sequelae, the results are similar, especially in the older age groups. In the younger age groups, both studies have low numbers of events and thus less confident estimates.

The credibility of such estimates increases when similar results are obtained by two different methods. The prevalence of stroke seems to be considerably higher than reported in older studies with more highly selected populations,⁵ and it increases from about 1% at age 50 to about 10% in the age group over 80 years. Approximately one in three of the younger patients and three in four of the older patients have persisting impairments and disabilities from the combined effect of stroke and other chronic diseases.⁶

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Response

It is encouraging to see an increasing number of well-designed population-based studies measuring the incidence rate and case fatality of stroke. However, determining the prevalence of stroke, a more useful measure for estimating the impact of stroke on community services and family caregivers, presents particular problems.

Increasing efforts are being made to validate self-reported history of stroke. As the Nord-Trøndelag study indicates, information elicited by self-report overestimates stroke prevalence by about one third.¹ Because many people recover from stroke, estimates that do not incorporate measures of ongoing disability related to stroke sequelae further overestimate the services required for stroke patients.

While it would be comforting to conclude that similar results between the Nord-Trøndelag study and the Auckland Stroke study reflect the true prevalence of stroke^{1,2} it is equally possible that the similarities could have occurred by chance. The lack of confidence intervals around the rates presented in the Table do

Prevalence Rates of Stroke and Stroke-Related Impairments/Disabilities (per 100 000 Population) in Nord-Trøndelag, Norway, and Auckland, New Zealand, by Age

Age Group	Nord-Trøndelag			Auckland		
	Overall	With Motor Impairment, n (%)	With Severe Motor Impairment, n (%)	Overall	Incomplete Recovery, n (%)	Needing Help in ADL, n (%)
40–49	230	62 (27)	18 (8)			
45–54				615	283 (46)	107 (17)
50–59	1010	384 (38)	162 (16)			
55–64				1649	751 (46)	344 (21)
60–69	2600	1170 (45)	520 (20)			
65–74				3772	2096 (56)	776 (21)
70–79	5690	3414 (60)	1536 (27)			
75–84				6681	4554 (68)	1533 (23)
80+	10 810	7675 (71)	4324 (40)			
85+				9530	7319 (77)	2948 (31)

ADL indicates activities of daily living.

not allow precise comparisons. In addition, differences in study design as well as definitions of disability also hamper comparisons. For example, the Auckland study used conservative cut points. Incomplete recovery included people who had no ongoing motor deficit; people requiring assistance in any one activity of daily living were included in the more disadvantaged group. The Nord-Trøndelag study included only patients with severe motor deficit as the more severe group. Although both studies have attempted to refine earlier stroke prevalence estimates, neither have succeeded in separating stroke-related disability from disability associated with other concomitant disabling conditions.

The challenge is for stroke epidemiologists to develop a consensus concerning the measures that best reflect stroke-related disability and how such definitions can be usefully incorporated into future studies in a standard manner. Dr Wyller and colleagues have contributed to the need for a debate on the issue.

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Causes and Mechanisms of Cerebellar Infarction in Young Patients

To the Editor:

The recent article by Barinagarrementeria and colleagues,¹ dealing with the causes and mechanisms of cerebellar infarction in young patients, is not only informative but also provocative. The finding that 67% were caused by vertebral artery dissection raises several questions. Specifically, the pathogenesis of spontaneous vertebral artery dissection remains unknown, yet several independent factors exist that may provide clues. Did any of the individuals have chiropractic therapy,² shampooing in a beauty parlor,³ or sustained neck angulation activity⁴ within a week prior to their stroke? It is well known that the mechanism of injury may be acute but also can be delayed, with intimal damage evolving over hours or days. Thus, the precipitating cause may be missed since physicians seldom ask about antecedent neck activities or prolonged angulation postures.

It is also well established that individuals harboring a hypoplastic vertebral artery are at augmented risk for brain stem stroke.⁴ Did any of the cohort have this congenital anomaly, since its presence imposes specific hemodynamic stresses? Last, a recent report⁵ implicated acute infectious disease as a cause of vascular injury and dissection. Did any of the cohort experience an infection within one week of onset?

In conclusion, physicians generally assume that the mechanisms of stroke are acute, yet intimal-medial defects or cumulative response to trivial traumas or infections need to be explored as part of a detailed neurological history.

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Response

We have read with interest the comments of Dr Weintraub. The frequency of vertebral artery dissection as cause of cerebellar infarction was 27%, explaining 10 of 15 cases (67%) with cerebellar infarction in territorial (PICA) distribution. There were no cases of dissection with history of chiropractic therapy, but two of our patients had a history of neck hyperextension with cervical pain in the week prior to stroke onset. As pointed out by Dr Weintraub, dissection may follow a variety of predisposing factors, including neck flexion or extension,¹⁻² peritonsillar trauma, and strangulation. We agree that these types of activities should be routinely researched in young patients with cerebral infarction.

In our series there were no cases with evidence of congenital anomalies in the vertebrobasilar system; neither was there a history of acute infectious disease in those patients with vertebral artery dissection.

It is well known that arterial dissection produce delayed ischemic symptoms. Many patients have headache or neck pain as first manifestation and develop ischemic symptoms hours or days later. Pain could be the only manifestation of arterial dissection.³

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Hypertension Control as an Indicator of the Geographical Variation of Stroke

To the Editor:

An interesting article in the *Stroke*¹ recently reported a correlation between the prevalence of high blood pressure (HBP) and the stroke attack rate in men ($r=0.42$, $P=.09$) and women ($r=0.56$, $P=.016$) aged 35 to 64 years across centers in the MONICA Project. These findings have prompted us to examine the relation between HBP control and stroke attack and mortality rates using data from the published reports of the MONICA project.¹⁻³

HBP control is defined as the proportion of actual hypertensives with systolic and diastolic blood pressures under 160 and 95 mm Hg, respectively. Data on age-adjusted HBP control are obtained from the first MONICA surveys in the mid-1980s,² while data on age-adjusted stroke attack and mortality rates correspond to the late 1980s.³ Finally, we have used data for the same MONICA centers as Stegmayr et al,¹ with the exception of the Novosibirsk control and the Gothenburg centers, because we could not extract the data from the MONICA reports.

Pearson correlation coefficients between HBP control and stroke attack rates were -0.29 ($P=.28$) for men and -0.18 ($P=.51$) for women. Correlations between HBP control and stroke mortality were -0.59 ($P=.01$) and -0.25 ($P=.34$) for men and women, respectively. From the perspective of the strategies of stroke prevention through HBP control, it is reassuring that this variable contributes modestly, although significantly among men, to the geographical variation of stroke across centers from very diverse countries.

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Autoimmunity in Down's Syndrome: Another Possible Mechanism of Moyamoya Disease

To the Editor:

The presence of Down's syndrome (DS) associated with moyamoya disease has been increasingly noted in the last years. Several reports suggest that the incidence of moyamoya disease is higher in children with DS than in other children. Since 1977, when this association was described for the first time, more than twenty cases have been reported.¹⁻³ However, the reason of this association is unknown. Furthermore, DS is associated with autoimmune disorders.⁴ We describe a child with trisomy 21 affected by moyamoya and Graves' disease, associated with anti-thyroid microsome antibodies and antiphospholipid antibodies (aPL). This patient was included in the prospective study of stroke in young adults in Cantabria, Spain.^{5,6}

A 21-year-old man was admitted to the hospital on May 27, 1986. Thirteen days before, his mother noticed a sudden muscle weakness in his left arm; 3 days later she also noted that he had difficulty in walking because of a weakness in his left leg. The patient was the eighth pregnancy of a mother who was 39 years of age at the time of delivery. When he was a baby, a mental deficiency was noted, and he also suffered from an incomplete acquisition of the language, with use only of monosyllables.

His blood pressure was 110/50 mm Hg and his pulse was 130. On general physical examination a mongoloid face, bilateral exophthalmos, and diffuse goiter were present. On neurological examination a moderate left hemiparesis with increased deep tendon reflexes of the left extremities was found.

Except for an increase of serum gamma globulin (23%), routine laboratory investigations were normal. A serologic test for syphilis and tests for antinuclear antibodies were negative. A lumbar puncture yielded normal cerebrospinal fluid. A x-ray examination of chest showed no abnormalities. An ECG and 24-hour Holter monitoring revealed sinus tachycardia at a rate of 130. An echocardiographic study demonstrated hyperdynamic systolic left ventricular function. A diagnosis of trisomy 21,

clinically suspected, was confirmed by karyotyping. Thyroid function tests showed the total thyroxine 21.2 $\mu\text{g/dL}$ (normal value, 4.0 to 12.5), the total T_3 583 $\eta\text{g/dL}$ (80 to 210), the resin T_3 uptake index 1.34 (0.85 to 1.15), the thyrotropin <0.5 mUI/L (0.15 to 4.5), negative antithyroglobulin antibodies, and positive antimicrosomal antibodies (titer of 1:400). The thyroid scintigraphy with $^{99\text{m}}\text{Tc}$ demonstrated a thyroid gland uniformly increased in size, with a regular distribution of the tracer. The coagulation studies revealed slight positive lupus anticoagulant (LA). Positive IgG anticardiolipin antibodies (ACA) were disclosed in plasma.

A CT scan of the cranial contents demonstrated a septum pellucidum cyst, slight calcifications in the basal ganglia, and right paraventricular frontal infarct. Right vertebral and internal carotid angiography showed marked stenosis of the supraclinoid portion of the carotid artery, important decreased flow in the middle cerebral artery, and nonvisualization of the anterior cerebral artery. Lenticulostriate arteries were prominently shown. The vertebrobasilar territory was normal, and there was a transcortical anastomosis from posterior cerebral arteries to anterior cerebral arteries. Left internal carotid angiography demonstrated a very poor visualization of anterior cerebral arteries.

A diagnosis of DS, Graves' disease, and moyamoya disease was established. The patient was treated with antithyroid agents and 250 mg acetylsalicylic acid daily. As a result of the cerebral infarction, the patient suffered a moderate disability in his left limbs. Antithyroid treatment was stopped in 1991; however, thyroid function continued to be normal from that point. Neither other cerebrovascular disorders nor other major diseases have since been detected in the patient. The last laboratory exams, which were carried out in January and August 1997, showed slight positive LA, positive ACA ELISA test (50 UGPL/mL), positive antimicrosomal antibodies (1:1000), and serum hypergammaglobulinemia of 31%.

This patient was diagnosed of Graves' disease and presence of thyroid autoantibodies. Patients with DS are known to have an altered immune system. In particular, they have an increased prevalence of autoimmune disorders, such as autoimmune thyroid disease.⁴ The high prevalence of thyroid autoantibodies found in DS patients (39.3%)⁴ supports the recognized association between thyroid dysfunction and DS.

Our patient also had aPL. The report of a woman with autoimmune hyperthyroidism and the presence of LA, antimicrosomal antibodies, and ACA has been described.⁷ Also reported has been the case of a child with trisomy 21 affected by hypothyroidism associated with antithyroglobulin and anti-thyroid microsome antibodies in the blood.⁸ Moreover, this patient was affected by multiple arterial thromboses in association with ACA, thus suggesting the antiphospholipid syndrome.⁸ This observed association does not seem a casual fact: on the contrary, it would possibly be related to unknown exogenous factors acting on subjects genetically predisposed to autoimmunity.⁸

Furthermore, our DS patient had clinical and radiological features consistent with the diagnosis of moyamoya disease. At present, the etiology of this disease is still unknown. It is not clear whether moyamoya disease represents a congenital arterial displasia or is a syndrome caused by nonspecific vascular reaction. Recently, it has been postulated that a protein encoded on chromosome 21 may be related to the pathogenesis of moyamoya disease.¹ However, the presence in trisomy 21 patients of autoimmune processes and autoantibodies make us suspect that the higher frequency of moyamoya disease in DS could be also result from an immune disturbance. Moreover, moyamoya disease itself has been associated with aPL.⁹

We conclude that different autoantibodies may be produced in DS. In subjects genetically predisposed to autoimmunity, these or other unknown antibodies could be associated with moyamoya

disease. We believe that testing for plasma aPL is important in DS associated with moyamoya disease.

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Homozygous C677T Mutation of the 5,10 Methylene-tetrahydrofolate Reductase Gene and Hyperhomocysteinemia in Italian Patients With a History of Early-Onset Ischemic Stroke

To the Editor:

Case-control^{1,2} and prospective^{3,4} studies have suggested an association between moderate hyperhomocysteinemia and risk of ischemic stroke. Homozygosity for the C-to-T substitution at nucleotide 677 of the gene of 5,10-methylene-tetrahydrofolate reductase (MTHFR) is associated with a 50% reduction of the activity of this enzyme⁵ and is the most common inherited cause of moderate hyperhomocysteinemia. In 1996 Kluitmans et al⁶ reported a threefold increase in the risk of early-onset cardiovascular disease in homozygotes for the C677T MTHFR mutation. However, the association of this genetic marker with arterial vascular events has been disputed by a nested case-control study.⁷ Markus et al⁸ recently failed to show an association between cerebrovascular disease and the MTHFR genotype, a comparable prevalence of homozygosity for the C677T MTHFR mutation being detectable in a population of 345 patients with ischemic stroke or transient ischemic attacks (TIA) and in 161 control subjects (10.7% versus 13.7%, respectively). Nor were nonfasting log homocysteine plasma levels able to identify subjects with a stroke history in that setting, as judged by the analysis of a subgroup of patients (n=160) and control subjects (n=75) in whom this amino acid was measured. However, as

Prevalence of Homozygous C677T MTHFR Mutation and Elevated tHcy Among Patients With a History of Early-Onset Ischemic Stroke and Control Subjects

	Patients (n=60)	Controls (n=182)
Homozygotes (+/+)*	22/60 (36.7%)	39/182 (21.4%)
tHcy (μM)†	15.8±14.6	12.5±7.8
Subjects with elevated‡ tHcy§	13/60 (21.6%)	18/182 (9.8%)

* $P=.035$ for patients vs controls (χ^2 test).

† $P=.004$ for patients vs controls (t test).

‡Elevated tHcy indicates mean plasma homocysteine levels above the 95th percentile of the distribution in age-matched control men (19.5 μM) and women (15 μM).

§ $P=.03$ for patients vs controls (χ^2 test).

expected, the authors found a significant relationship between MTHFR genotype and homocysteine levels, the latter being also independently related to log serum folate.

In the frame of a larger study on juvenile thrombotic events, we have evaluated a population of 60 consecutive patients with a history of early-onset ischemic stroke (29 females and 31 males, aged 5 to 64 years [mean age, 38; mean age at time of diagnosis, 34; range 4 to 49 years]) documented within 72 to 96 hours from the event by CT and/or MRI scans. Subjects who had suffered from TIA or who exhibited abnormalities of carotid and/or vertebral arteries were excluded from the study. As many as 182 subjects matched for sex and age, without a history of thrombosis, served as controls. Total fasting plasma homocysteine (tHcy), ie, the sum of free and protein-bound forms plus cysteine-homocysteine mixed disulfide, was measured by isolation of the amino acid by high-performance liquid chromatography and fluorescence detection.⁹ Hyperhomocysteinemia was defined as tHcy values above the 95th percentile of the distribution within the general population (>19.5 μM for males and >15 μM for females in our population).⁹ The C677T MTHFR mutation was studied by endonuclease digestion with *Hinf*I of the polymerase chain reaction-amplified products.¹⁰ As summarized in the table, among patients the prevalence of homozygous C677T MTHFR mutation (+/+) was 36.7% (22 of 60); among control subjects, it was 21.4% (39 of 182 individuals; $P<.05$, χ^2 test). Elevated fasting tHcy was detected in 13 of 60 patients (21.6%) and in 18 of 182 control subjects (9.8%) ($P<.05$, χ^2 test). Mean tHcy was 15.8±14.6 μM in patients and 12.5±7.8 μM in controls ($P<.005$, t -test). By analyzing the distribution of hyperhomocysteinemic patients among the different MTHFR genotypes, a significantly higher prevalence of elevated tHcy was found among +/+ homozygotes compared with other genotypes. Nine of the 22 +/+ patients (40.9%) showed fasting plasma homocysteine levels above the 95th percentile of the distribution. Only 4 of the 38 (10.5%) nonhomozygotes (+/- and -/-) behaved similarly ($P<.005$ by the χ^2 test, homozygotes versus nonhomozygotes). Accordingly, higher mean fasting tHcy was detected in +/+ homozygous patients than in nonhomozygotes (23.3±22.2 μM versus 11.8±4.9; $P<.005$). Similar to the findings of Markus et al,⁸ plasma tHcy was inversely related to folate levels ($r=-.26$; $P<.001$) in this setting. Folate plasma levels did not differ among patients and controls. In a logistic regression model, the homozygous C677T MTHFR mutation was significantly associated with the event (odds ratio, 2.1; 95% confidence interval, 1.1 to 4.0; $P=.02$). By excluding the C677T MTHFR mutation from the model, the association with tHcy became significant (odds ratio, 1.03; 95% confidence interval, 1.001 to 1.06; $P=.04$). Accordingly, a multiple regression analysis showed that C677T MTHFR mutation was a major determinant of tHcy (β coefficient=8.67; $P<.001$).

Several concepts should be taken into account in understanding differences between the present findings and those of Markus

et al. In our setting, only individuals with juvenile stroke were enrolled. Patient mean age in the study of Markus et al was 65.7 years. An unknown proportion of patients with TIA was evaluated in that report; patients with TIA were excluded from our study. As a whole, in our study fasting hyperhomocysteinemia and the MTHFR mutation allowed identification of almost 40% of subjects with a history of stroke. It is known that nonfasting plasma homocysteine levels help to identify higher numbers of thrombophilic subjects with this metabolic abnormality.⁹ However, factors other than the C677T MTHFR mutation play a role in regulating nonfasting homocysteine levels. The strong linkage between C677T homozygosity and fasting hyperhomocysteinemia in this setting further supports this association. The high frequency of the mutation in our setting may be the result of an inappropriate sample selection and may have greatly contributed to the significant and independent association between the genetic marker and ischemic stroke reported here. However, figures on the frequency of the C677T homozygous state in Italian populations are comparable with those reported by our and other groups,^{10,11} and the combined data are consistent with the possibility that genotype-based observations can be applied only to ethnic groups of similar genetic background.

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Response:

We were interested to see the results of this study in an Italian population which suggests that the C677T MTHFR gene polymorphism may be a risk factor for early-onset stroke. Homocysteine levels were measured using a methodology similar to that in our study but these were fasting levels in contrast to the levels we measured, which were nonfasting and performed only in a subgroup of patients. Consistent with their data and with previous studies, we found that homocysteine levels were higher in subjects than in controls, but this result did not reach significance (mean [SD] log homocysteine level in cases, 1.32 [0.19] mmol/L; in controls, 1.27 [0.19] mmol/L; $P=.09$). This lack of significance is likely to result from small sample numbers (homocysteine was measured only in a subgroup) and from a weakening of any association by the use of nonfasting levels. Nevertheless, this result is consistent with the existing literature and the results of Soriente et al on an association between raised homocysteine levels and stroke risk. In contrast, there was not even a trend toward an increase in the prevalence of homozygosity (*TT*) for the C677T MTHFR polymorphism in our population (*TT* genotype frequency in cases was 10.7% versus 13.7% in controls; $P=.34$) or in *T* allele frequency (0.68 versus 0.67; $P=.67$). This suggests that in our population, which was an unselected group of consecutive white patients presenting with ischemic stroke and TIA, the MTHFR genotype is not a major risk factor for stroke. As mentioned in our article, we also performed subgroup analysis to determine whether the polymorphism was a risk factor for patients with stroke compared with patients with TIA and no CT infarct, but there was no difference in *TT* frequency between the two groups (11.1% versus 9.5%; $P=.69$). In addition, there was no association between genotype and cerebrovascular disease when only the 157 case subjects and 79 control subjects aged <65 years, were considered (*TT* frequency of 13.5% in case subjects and 15.2% in control subjects; $P=.70$). Unfortunately, because of an error at the editing stage, this line was printed as ≥ 65 years rather than the ≤ 65 years in the original manuscript. The majority of studies to date looking at the association between the MTHFR polymorphism and cardiovascular risk, most of which have looked at ischemic heart disease, have also failed to show a strong association.

Therefore, the results of Soriente et al, which show a strikingly increased *TT* genotype prevalence in stroke patients, are at variance with ours. However, the association, once other risk factors had been accounted for in a logistic regression model, was only just significant, with a lower confidence interval of 1.1. These results may reflect a real difference between the two populations. The patients they studied were young, with a mean age of 34 and a youngest age of 4, which is significantly lower than both the mean age of our patient population and the mean age of our subgroup of patients aged ≤ 65 years. Another important factor could be the folate intake of the population. Both our study and that of Soriente et al show a significant interaction between serum folate, MTHFR genotype, and homocysteine levels. Therefore, if the folic acid intake of their population is lower than that of ours, the MTHFR genotype will have a greater effect on determining serum homocysteine levels. An alternative explanation is that this is a chance finding, which can frequently happen with candidate gene association studies. Only 60

patients were studied. No information is given about how the control population was recruited or whether the study individuals were ethnically homogenous. In addition, a relatively high frequency of the *TT* genotype was found both in cases and controls.

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Interrater Agreement on a Simple Neurological Score in Rats

To the Editor:

Strong and reliable outcome measures are required in laboratory studies that aim to appraise the extent of the damage in animals subjected to various forms of cerebral ischemia. In this regard, the assessment of histological changes, such as the volume of infarcted tissue or the number of necrotic cells, is considered the gold standard. However, the assessment of functional outcome can also be useful in animal studies that evaluate the effect of new therapeutic agents, since the clinical examination is effortless and not time demanding; moreover, physical testing of the animals can be repeated over time and thus provide data on the evolution of the neurological deficit. A simple neurological score to evaluate sensorimotor performance in rats has recently been developed by Garcia et al.¹ It explores six different functions and attributes to each a 3- or 4-point score. The total score, which correlates closely with the severity of the histological injury (in particular with the number of necrotic neurons) in a model of middle cerebral artery (MCA) occlusion in the Wistar rat,¹ has been used as one of the outcome measures in studies that estimate the effect of new drugs.^{2,3} Because of its simplicity and strong correlation with histological damage, this score might be used in other laboratories employing experimental procedures to cause cerebral ischemia. In this regard, evaluation of its reproducibility could be extremely useful, because it is known that the validity of scales evaluating neurological deficit can be affected by interobserver variability.^{4,5}

We have conducted a study to evaluate the impact of personal judgment in the use of this scale and to explore which of the six different functions might be particularly affected by interobserver variability. Thirty-one male Charles River Wistar rats weighing 270 to 310 g were studied. Twenty rats had permanent occlusion of the right MCA through use of an intraluminal filament,⁶ 2 had a sham operation for MCA occlusion (as in the preceding group but with the filament withdrawn within 30 seconds), 3 underwent ligation of the right common carotid artery, 3 underwent a bilateral ligation of the common carotid artery, 2 had a sham operation for carotid artery

ligation (ie, exposure of the arteries in the neck), and 1 was a normal control. All the surgical procedures were carried out under general anesthesia with halothane.

Two observers consecutively and independently carried out the neurological examination of each rat 1 to 3 days after surgery, according to published guidelines.¹ The investigators, both experienced in laboratory procedures involving animals, were blinded to the surgical procedure that the animals had undergone, with the exception of the normal control animal (who was easily identifiable by absence of skin incision). To measure the level of interobserver agreement, we used the weighted κ coefficient, based on a formula proposed by Cohen.⁷ Conventionally, κ values are considered as follows: 0.01 to 0.20, slight; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, substantial; and 0.81 to 0.99, almost perfect.⁸ (A value of +1 indicates perfect agreement.) The statistical significance was evaluated by means of 95% confidence limits.

Considering the entire group of animals, the interobserver agreement was substantial for each of the items and almost perfect for total score. When the analysis was restricted to the rats with permanent MCA occlusion (ie, those in which sensorimotor deficits were present), the agreement worsened, in particular on the items "movements symmetry" and "body proprioception"; the agreement on the total score remained substantial (Table).

Whereas previous papers correlated the extent of neurological deficit following disparate cerebral ischemic procedures with the area or volume of infarct,^{9,10} the sensorimotor score developed by Garcia et al¹ shows a strong correlation with the number of necrotic neurons in rats with permanent or transient MCA occlusion. Therefore, it might also represent a useful tool for the assessment of functional outcome in animals with limited ischemic brain damage. Testing by this method does not require training of the rats or purchase of expensive equipment. In addition, results can be expressed in a numerical score compatible with analysis by statistical means. In the original article¹ the extent of rat neurological deficit was assessed by two raters, but data on interobserver variability were not provided. Interrater reliability is an essential requirement for a scale that measures functional outcome in neurological assessment. Differences reported can be accounted for by the interrater variability instead of true variations in the observed phenomenon. This has been emphasized in studies evaluating interobserver variability in the neurological examination of stroke patients.^{5,11} Our study shows that the reliability of this brief rat neurological scale is fair, although not completely free from interrater variability, particularly in such items as movements symmetry and body proprioception. We believe that this variability may partly depend on

Interobserver Agreement on Each of the Six Sensorimotor Functions and Total Score of the Scale of Garcia et al¹

Function	Total Sample (n=31)		Rats With Permanent MCAO (n=20)	
	Weighted κ	97% CL	Weighted κ	95% CL
Spontaneous activity	0.70*	0.53-0.87	0.65*	0.44-0.87
Movements symmetry	0.75*	0.58-0.91	0.49‡	0.18-0.79
Forepaw outstretching	0.61*	0.27-0.94	0.46‡	0.02-0.90
Climbing	0.74*	0.52-0.96	0.66*	0.36-0.97
Body proprioception	0.65*	0.39-0.91	0.53‡	0.08-0.98
Response to vibrissae touch	0.67*	0.44-0.90	0.59‡	0.24-0.92
Total score	0.87†	0.78-0.96	0.79*	0.61-0.96

MCAO indicates middle cerebral artery occlusion; CL, confidence limits.

*Substantial agreement; †almost perfect agreement; and ‡moderate agreement (according to Landis and Koch⁸).

dissimilar behavior of the animals in different moments rather than on raters' variability. This flaw is in part lessened by the substantial agreement on the total score that is the only score finally used.

Future studies based on the premise that a certain agent is effective in reducing the ischemic insult to the brain parenchyma should be based on (1) the knowledge of the chronology and topography of the lethal neuronal injury as it exists in the absence of therapeutic intervention; (2) a measure of the degree of the neurological deficit induced by the injury; and (3) a verification of the close relationship between (1) and (2). Based on the above-reported characteristics of the scale and its rather fair reliability, we suggest that the score of Garcia et al may be used by researchers to evaluate the functional outcome of rats undergoing a variety of experimental procedures aimed at provoking an insult to the brain parenchyma that results in a sensorimotor deficit.

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Response

The study by Pantoni and associates described above adds an important evaluation of the interrater agreement on the method to assign a neurological score originally designed to determine whether there exists a reliable correlation between the extent of sensorimotor deficit (induced in rats by a middle cerebral artery occlusion) and the numbers of necrotic neurons identified by histological methods 7 days after the original injury.¹ From these and additional studies we emphasize the following important issues.

(1) In this species (male Wistar rats from Charles River; identified as Cr1 (WI)Br), *permanent* occlusion of one middle cerebral artery induces pannecrosis (infarction) of the entire arterial territory only 3 to 4 days after the injury.²

(2) Necrotic neurons (defined by histological criteria) appear quickly (within 12 hours) in the striatum, while in the cortex large numbers of necrotic neurons become identifiable only several (2 to 3) days later.³

(3) Reopening the artery after 60 minutes induces a brain lesion whose features are remarkably different from those of the infarction (pannecrosis) that develops after 4 days in all rats in which the artery was not reopened.⁴

(4) The "area of pallor" that becomes visible in sections stained with hematoxylin-eosin 24 to 48 hours after a *permanent* arterial occlusion never appears in brains subjected to *transient* arterial occlusion.^{2,4} Thus, under these conditions it is essential to evaluate numbers of necrotic neurons as a logical end point.

(5) In experiments based on short-term (<30 minutes) arterial occlusions followed by long-term (up to 28 days) reperfusion, there is a lapse of 3 to 4 days between the time of the injury and the appearance of necrotic neurons in the cortex.⁴ This emphasizes once more the wisdom or the necessity of relying on counts of necrotic neurons as an end point that accurately reflects the degree of brain injury caused by arterial occlusions of variable duration.

We are pleased to learn that an independent laboratory has verified the reliability of this simple method to evaluate sensorimotor responses in the rat.

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