

Cerebrospinal fluid enolase in stroke

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SUMMARY This study relates the level of α and γ enolase in cerebrospinal fluid sampled within 4 days of a stroke to the volume of the cerebral infarct measured on the CT image and to the clinical outcome of the patient. Twenty-eight patients were studied, two with transient ischaemic attacks and 26 with completed stroke due to infarction. The cerebrospinal fluid enolase was raised in the two patients with transient ischaemic attacks and 23 with completed stroke. There was a positive correlation between the volume of the infarct and the level of cerebrospinal fluid α and γ enolase. A high cerebrospinal fluid enolase was always associated with a poor prognosis.

There have been several studies measuring serum and cerebrospinal fluid enzymes following cerebrovascular accidents. Enzymes studied include creatine kinase, glutamic oxaloacetic transaminase, lactic dehydrogenase, aldolase, adenylate kinase and enolase.¹⁻¹³ Creatine kinase was investigated as a possible indicator of damage since it is present in high concentrations within the brain.¹⁰ However, Nathan¹³ found raised cerebrospinal fluid creatine kinase in only two of five patients with completed strokes and in no patients following a transient ischaemic attack. Raised serum creatine kinase has been shown to consist predominantly of the muscle type isoenzyme (CK MM) and not the brain type isoenzyme (CK BB).⁸

Royds *et al*⁴ measured the total activities of aldolase, enolase, pyruvate kinase, lactic dehydrogenase and creatine kinase in the cerebrospinal fluid of 121 patients with disorders of the central nervous system and concluded that of these five cerebrospinal fluid enzymes, enolase was the most sensitive marker of central nervous system disease.⁴

Enolase is a dimeric enzyme which catalyses the interconversion of 2-phospho-D-glycerate and phosphoenolpyruvate in the glycolytic pathway. It has three immunologically distinct subunits α , β , and γ , giving rise to the five isoenzymes $\alpha\alpha$, $\beta\beta$, $\gamma\gamma$, $\alpha\beta$, and $\alpha\gamma$. Within the nervous system glial cells contain

only $\alpha\alpha$ enolase, and $\gamma\gamma$ enolase is confined to the neurons.¹⁴ Using an immunoperoxidase peroxidase anti-peroxidase (PAP) staining technique, Royds *et al*⁴ have shown that ischaemic neurons stain weakly or not at all for enolase, the enzymes presumably being released into the extracellular space and then the cerebrospinal fluid.

Royds *et al*¹⁵ reported that both α and γ enolase are raised in the cerebrospinal fluid of patients following strokes and in the majority of patients following a transient ischaemic attack and suggested that there might be a useful relationship between the isoenzyme levels and the extent of brain damage. The present study explores this hypothesis by measuring immunoreactive α and γ enolase in serum and cerebrospinal fluid from patients following a cerebrovascular accident in relationship to the volume of infarcted tissue as measured on computed tomography (CT) and the clinical outcome.

Patients and method

Twenty-eight patients were studied with evidence of non-haemorrhagic acute cerebrovascular disease. There were 18 men and 10 women, mean age 63.4 years (range 44-80 years). The diagnosis of stroke was based upon the occurrence of an episode of focal neurological disorder of acute onset producing hemiparesis or hemiplegia with or without hemisensory impairment, hemianopia or aphasia persisting for longer than 24 hours. Two of the 28 patients fitted the definition of transient ischaemic attacks. Patients with other proven neurological disease were excluded from the study, as were patients with cerebrospinal fluid containing red cells or demonstrating xanthochromia. Patients were assessed as soon as possible after admission to hospital and

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the findings recorded. Within 4 days a lumbar puncture was performed. The timing of this was unavoidably variable owing to a number of factors such as delay of admission or delay of referral. The cerebrospinal fluid was immediately examined for cells and protein and stored at -16°C until the enolase assay was performed.

Radioimmunoassay of α and γ immunoreactive enolases

This was performed as previously described.¹⁵ Radiolabelled $\alpha\alpha$ and $\gamma\gamma$ enolases were prepared by the method of Bolton and Hunter¹⁶ using N-succinimidyl-3, 4 hydroxy-5 (¹²⁵I) iodophenyl propionate (Amersham International Ltd, Amersham, Bucks HP7 9LL UK). The primary antiserum was produced in rabbits by injections of purified antigens.¹⁴ The assays were carried out in Luckham LP3 tubes (Luckham Ltd, Victoria Gardens, Burgess Hill, Sussex RM15 9QN UK) and the separation of bound and free antigen in the assay was accomplished by precipitation using goat anti-rabbit IgG (Miles Laboratories Ltd, Slough, Bucks). Purified samples of $\alpha\alpha$ and $\gamma\gamma$ enolase prepared as previously described were used as standards in the assay.¹⁴

One week following the onset of symptoms a CT scan was performed on each patient. The volume of the cerebral infarct was measured using the Diagnostic Enhancement Package by EMI Ltd.¹⁷ With this software package the perimeter of the infarct can be traced with a light pen and

from this the area on each slice is calculated by the computer. The volume in each slice is thus this area multiplied by the slice thickness. This process is repeated on sequential slices traversing the whole of the infarct. The total volume is therefore the sum of the volumes on each relevant slice. The main error in this technique is in defining the border of the lesion. This is derived by taking the mean of the densities of the infarct and of the surrounding brain. It is traced at a narrow window width of 20 Hounsfield units. After several phantom experiments an accuracy of volume estimation of $>90\%$ was obtained.

Three months following the cerebrovascular accident, the patients were reassessed and persisting disability was graded in accordance with the Glasgow Outcome Scale of Jennet and Bond.¹⁸

Results

Table 1 shows the values for CSF immunoreactive α and γ enolase and the volumes of cerebral infarction for 28 consecutive patients presenting with non-haemorrhagic cerebrovascular disease. CSF samples from 23 patients who had no organic neurological disease or who were undergoing myelography were used as controls. The mean values for immunoreac-

Table 1 CSF concentrations of immunoreactive α and γ enolase and infarction volumes in 28 patients with non-haemorrhagic cerebrovascular disease

Patient	Sex	Age (yr)	Volume of infarct. ml.	α enolase ng/ml	γ enolase ng/ml	$\alpha + \gamma$ enolase ng/ml	Time of lumbar puncture (days)	Outcome
JP	M	71	*—	1000	70	1070	2	Death
RM	M	48	116	620	140	760	3	Severe disability
JD	M	60	92	179	56	235	1	Severe disability
JC	F	76	32	184	43	227	1	Severe disability
DM	F	52	62	125	45	170	2	Severe disability
MJ	F	68	42	68	58	126	½	Severe disability
JS	M	70	*—	70	32	102	2	Death
GB	M	75	23	73	47	120	½	Severe disability
SP	M	43	57	71	26	97	1	Severe disability
HH	M	78	86	62	33	95	2	Severe disability
CW	M	70	25	50	20	70	1	Severe disability
TO	M	64	7	29	24	53	1	Severe disability
MC	F	80	7	39	12	51	1	Severe disability
TR	M	68	—	47	3	50	1	Vegetative
ES	M	72	—	33	14	47	1	Moderate disability
ES	F	68	—	23	19	42	1	Good recovery
RP	M	66	30	33	9	42	1	Good recovery
RS	M	58	—	20	22	42	1	Moderate disability
RMc	M	70	—	19	20	39	1	Good recovery
JMc	M	51	18	19	15	34	1	Good recovery
MJ	F	58	2	20	14	34	1	Good recovery
MB	F	50	15	20	12	32	3	Severe disability
GB	M	72	—	13	11	24	1	Good recovery
MN	F	54	7	12	9	21	2	Good recovery
SW	M	66	—	10	8	18	1	Severe disability
MT	F	80	—	12	4	16	1	Good recovery
JR	F	50	7	8	6	14	4	Good recovery
DH	M	44	—	9	3	12	2	Good recovery

*CT Scan not performed

Control values for enolase isoenzymes were derived from 23 patients with no organic neurological disease or who were undergoing myelography. Maximum normal values (mean \pm 2 \times SD) are 10.4 ng/ml for immuno-reactive α enolase, and 8.0 ng/ml for immunoreactive γ enolase. The results for the patients are arranged in descending order of α plus γ enolase values to facilitate assessment of correlation with ultimate outcome.

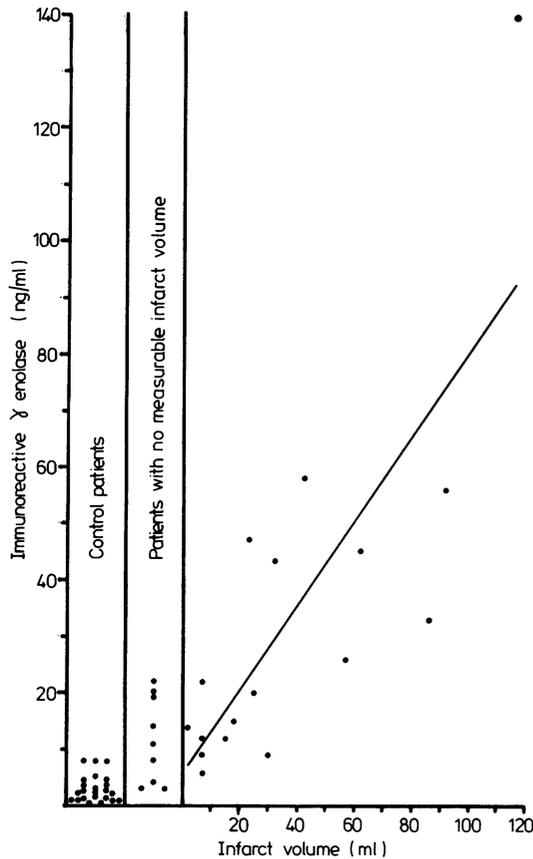


Fig 1 The relationship between CSF immunoreactive γ enolase and the volume of cerebral infarction. Enolase values for patients diagnosed as CVA by objective means but showing no measurable infarct volume by CT scan were entered in a separate column and not as zero volume on the regression diagram. The statistical parameters for these data are given in table 2.

tive α and γ enolase in this group are 5.0 ± 2.7 (SD) ng/ml and 3.0 ± 2.4 (SD) ng/ml respectively.

The results were examined for correlations between the volume of cerebral infarction and α , γ , or α plus γ immunoreactive enolase. The correlation for γ versus volume is shown in fig 1 and the statistical data for all the correlations is given in table 2.

CSF immunoreactive α and γ enolases both increased with volume of infarction, the regression coefficients being highly significant ($p < 0.001$). There was a significant correlation between the

increase in α and γ enolase values ($p < 0.001$), the α enolase rising on average 4.8 times as fast as the γ enolase.

Figures 2a and 2b show the immunoreactive α plus γ enolase values and the volumes of cerebral infarction in relation to clinical outcome as measured by the Glasgow Outcome Scale of Jennett and Bond.¹⁸

It is interesting to note that in the most severe case, which had a fatal outcome (JP, table 1), the ratio of α : γ in the CSF was 14.3:1 much nearer to

Table 2 Parameters of regression and correlation involving CSF enolase isoenzyme levels and stroke volumes

Comparison $n = 17$	Slope	r	p
Stroke volume (x) vs Immunoreactive α enolase (y)	3.18	0.75	<0.001
Stroke volume (x) vs Immunoreactive γ enolase (y)	0.74	0.79	<0.001
Stroke volume (x) vs Total immunoreactive $\alpha + \gamma$ enolase (y)	3.9	0.76	<0.001
Immunoreactive α enolase (x) vs Immunoreactive γ enolase (y)	0.21	0.94	<0.001

CSF immunoreactive α and γ enolase levels were measured in ng/ml. Cerebral infarction volumes were measured in ml as described in Methods. Only patients who had a measurable infarct volume on the CT scan were included in the calculations.

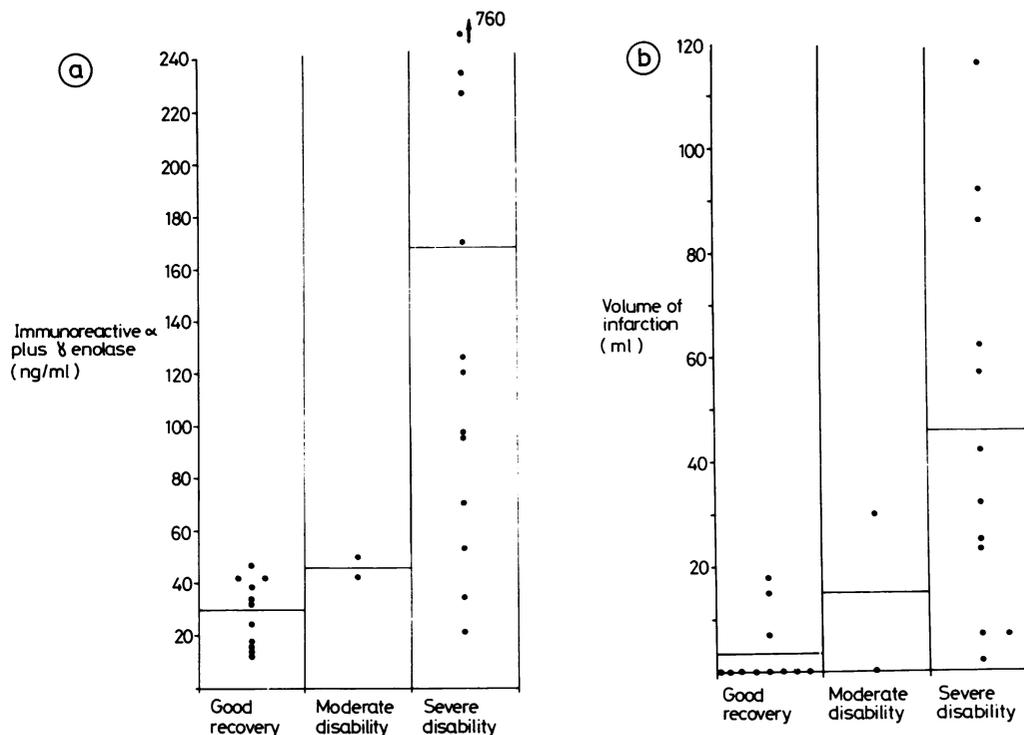


Fig 2 (a) Total immunoreactive α plus γ enolase levels in CSF; results grouped according to clinical outcome (b) cerebral infarction volume in relation to the clinical outcome.

the ratio existing in normal serum 20–30:1¹⁹ (authors' observations), than for normal CSF (1:1) suggesting damage to the blood-brain barrier. The α : γ ratios in most of the other CSFs approximated more closely the ratios found in cerebral tissues which vary between 3.7:1 and 1:1 according to the area of brain examined.²⁰ These results support the notion that in the majority of cases the increased CSF enolase following a stroke is derived from cerebral tissue rather than the serum.

Discussion

There has been considerable divergence of opinion as to the clinical significance of raised serum and cerebrospinal fluid enzymes in patients with acute cerebrovascular disease. Lactic dehydrogenase, glutamic oxaloacetic transaminase and aldolase activities in serum and cerebrospinal fluid following strokes have been found to be inconsistent markers of cell damage.^{2,3,10}

Terent and Ronquist¹ compared the cerebrospinal

fluid activities of adenylate kinase in seven patients with global cerebral ischaemia and 21 patients with strokes. The mean adenylate kinase activity was significantly higher in patients with global cerebral ischaemia than in those with strokes, suggesting a correlation between the degree of release of the enzyme and the extent of brain ischaemia.

Somer *et al*⁶ measured serum total creatine kinase (CK) and brain type creatine kinase (CK BB) in 12 patients with strokes and found raised CK BB in eight of these within a few hours of onset. There was no correlation between the levels of CK BB and total CK. The need for such early sampling considerably reduces the usefulness of this test. Further work by Kaste *et al*⁵ suggested that the detection of CK BB in the serum following a stroke was associated with a poor prognosis.

The sensitivity of cerebrospinal fluid enolase as a marker of brain damage has been previously shown by the raised levels found in patients with transient neurological disorder. Immunoreactive α and γ enolase are raised in the majority of patients with

epilepsy and transient ischaemic attacks and approximately 50% of patients with migraine.¹⁵

The results of the present study confirm the sensitivity of cerebrospinal fluid enolase as a marker of cerebral ischaemia or infarction. There was a positive correlation between the volume of the infarct measured on the CT scan (and hence the amount of infarcted tissue) and the levels of cerebrospinal fluid immunoreactive α and γ enolase. Although the neurological deficit depends on the site as well as the size of the infarct, the level of cerebrospinal fluid enolase was, however, related to the clinical outcome. A high level of total cerebrospinal fluid enolase (>50 ng/ml) was invariably associated with a poor prognosis in our patients, whilst all the patients making a good recovery had a total cerebrospinal fluid enolase <50 ng/ml. Two patients with total cerebrospinal fluid enolase of less than 50 ng/ml were left with severe disability. In both these a capsular infarct was seen on the CT scan, illustrating that a small strategically placed infarct can lead to substantial clinical deficit.

Two patients had particularly high total cerebrospinal fluid enolase (JP, 1070 ng/ml, RM 760 ng/ml). The former subsequently died and the volume of the infarct was not obtained because he was too restless for a diagnostic CT scan during life and permission for necropsy was refused. The other patient had a very large cerebral infarct of 116 ml and remains severely disabled.

Four patients had an infarct volume of 7 ml with differing levels of cerebrospinal fluid enolase. The explanation for this may lie in the timing of the lumbar punctures. The two patients whose lumbar punctures were performed within 24 hours had the highest levels with α 38.8 ng/ml, γ 11.7 ng/ml and α 29 ng/ml, γ 24 ng/ml respectively. In the third patient, the lumbar puncture was performed at 2 days and the levels were α 12.1 ng/ml, γ 9.3 ng/ml. The fourth patient had cerebrospinal fluid enolase levels of α 12.1 ng/ml, γ 5.9 ng/ml; the specimen was obtained 4 days following the stroke. It is not known how soon raised enolase levels can be detected in the cerebrospinal fluid following a stroke or for how long they remain elevated. These results suggest, as already demonstrated in animals, that enolase is rapidly cleared from human cerebrospinal fluid.²¹

In no patient was the cerebrospinal fluid enolase levels normal when a cerebral infarct was present on the CT scan.

Very high α and γ enolase levels were found in the cerebrospinal fluid of one patient who was excluded from the study on the basis of her CT scan (α 180 ng/ml, γ 100 ng/ml). Clinically she was demented with a right hemiparesis and her CT scan showed multiple infarcts. It was concluded that she had

multi-infarct dementia and the recurring destruction of cerebral tissue was responsible for the grossly elevated cerebrospinal fluid enolase levels.

In all patients the CT scan was performed 1 week following the onset of symptoms as by 7 to 10 days the infarct becomes better defined on the CT image with lower attenuation values than in the acute stage.²² A cerebral infarct was not visualised on the CT scan in seven of 26 patients with completed stroke and both patients with transient ischaemic attacks. Alcalá²² reported a series of 40 cerebral infarcts of ischaemic and haemorrhagic types in which 19 were positive and 21 negative on the CT scan. Size was the main factor governing the visualisation of the infarct as 12 of 13 infarcts less than 2 cm diameter were not visualised compared with two of 11 infarcts greater than 5.1 cm diameter. He also suggested that admixture of infarcted and haemorrhagic tissue could lead to a lesion isodense with surrounding brain and hence not detectable on the CT image.

This study shows correlations between enolase isoenzyme levels in CSF and the volume of cerebral infarction. The values of both enolase levels and infarction volume are related to the clinical outcome.

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