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Coagulation and fibrinolytic activity of cerebrospinal fluid

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SUMMARY Fibrin/fibrinogen degradation products (fragments D and E) were detected in cerebrospinal fluid in 23·4% of 252 patients admitted to a neurological/neurosurgical unit. Other coagulation proteins of low molecular weight (plasminogen and factor IX) were also present but larger proteins (fibrinogen and factor V) were not. These findings are consistent with protein leakage across a blood-CSF barrier damaged by inflammatory, vascular, or neoplastic disease. Fibrin/fibrinogen degradation products in cerebrospinal fluid after subarachnoid haemorrhage may not, therefore, be a reliable index of increased fibrinolytic activity in the subarachnoid space and may be misleading when selecting patients for fibrinolytic blockade.

Proteins with coagulant or fibrinolytic activity are not normally detected by conventional bioassay methods in unconcentrated cerebrospinal fluid (CSF). Nevertheless, it is a well-known clinical observation that CSF specimens may clot in pathological conditions such as tuberculous meningitis. The products of fibrinolytic activity—fibrin/fibrinogen degradation products (FDP)—have been found in CSF after subarachnoid haemorrhage (Tovi, 1972; Tovi *et al.*, 1972), in meningitis (Brueton *et al.*, 1974, 1976), and in 18·5% of patients admitted to a district psychiatric unit (Hunter *et al.*, 1974).

Relatively little is known of the underlying mechanisms whereby FDP and other coagulation-fibrinolytic proteins appear in CSF. It has been assumed that the high CSF level of FDP after subarachnoid haemorrhage results from lysis of fibrin deposited around the bleeding point (Tovi, 1972; Tovi *et al.*, 1972), but the expected increase in CSF plasminogen activator has either not been detected (Tovi *et al.*, 1972) or only slight activity has been detected in an occasional patient (Tovi *et al.*, 1973). Nevertheless, therapeutic inhibition of the presumed excess fibrinolytic activity of CSF has been recommended to prevent rebleeding after subarachnoid haemorrhage. Clinical studies have given encouraging results (Mullan and Dawley, 1968; Ransohoff *et al.*, 1972; Nibbelink *et al.*, 1975; Sengupta *et al.*, 1976) and a controlled clinical trial is currently in progress (Maurice-Williams, 1977). In meningitis, in contrast, enhancement of CSF fibrinolytic activity using streptokinase and urokinase has been advocated for the removal of excess fibrin (Stewart, 1964; Newman and Stewart, 1965).

The present study was undertaken to determine the conditions in which these coagulation-fibrinolytic proteins appear in CSF, to assess their diagnostic value, and to investigate their origin.

Patients and methods

Cerebrospinal fluid was obtained by lumbar, cisternal, or ventricular puncture done for diagnostic or therapeutic purposes in 252 patients admitted to a neurological/neurosurgical unit. The CSF (1·0 ml) was collected directly into 0·1 ml of 3·13% sodium citrate in a plastic tube for measuring factor V (Wolf, 1953), factor IX (Hardisty and Ingram, 1965), plasminogen (De Vreker, 1965), and fibrinogen (Diagen latex slide test; Diagnostic Reagents Limited). The specimens were transported at 0-4°C to the laboratory and centrifuged immediately at 1600 *g* for five minutes at 4°C. An additional 1·0 ml of CSF was collected into aprotinin (250 units), and 0·1 ml thrombin (final concentration 20 units/ml) was added before incubation at 37°C for one hour followed by centrifugation and assay of the supernatant for FDP (Thrombo-Wellcotest; Wellcome Reagents Limited).

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Plasminogen activator was estimated by adding citrated CSF to unheated fibrin plates (Astrup and Müllertz, 1952; Cash and Allan, 1967) within one hour of collection. Red cell counts in CSF were performed using an improved Neubauer counting chamber and CSF total protein was estimated by the method of Meulemans (1960) and Pennock *et al.* (1968). Statistical significance was determined by correlation coefficient and Student's *t* test.

Results

Twenty-two patients who proved to be clinically normal with no neurological abnormality had normal concentrations of CSF coagulation proteins (Table 1). In the study of 252 patients (Table 2) FDP were detected in the CSF in 59 (23·4%). Out of these 59 all but one also had a raised CSF protein, 42 had plasminogen in the CSF, and 26 had factor IX in the CSF. The larger molecular weight factor V and fibrinogen were not detected in any of the specimens.

Whenever thrombin-treated CSF gave a negative reaction with the Thrombo-Wellcotest antibody (that is, no detectable fragments D and E) the corresponding citrated CSF specimen without thrombin always gave a negative reaction with the Diagen reagent (that is, no detectable fibrinogen). Whenever thrombin-treated CSF gave a positive reaction with the Thrombo-Wellcotest antiserum the corresponding citrated specimen gave either a weaker or a negative reaction with the Diagen test. This was also interpreted as an absence of detectable fibrinogen in view of the greater sensitivity of the latter test to fibrinogen as opposed to fragments D and E (Donati *et al.*, 1973). These authors similarly used the combined technique to differentiate fibrinogen-related material in urine.

The patients were divided (Table 2) into clinical conditions in which increased permeability of the meningeal and choroidal capillaries with loss of integrity of the blood-CSF barrier might be expected (group 1) and conditions in which the barrier would normally be intact (group 2) (Schliep and Felgenhauer, 1974).

GROUP 1

Ten of the 13 patients with acute subarachnoid haemorrhage were studied within 96 hours of clinical onset and all showed two or more low molecular weight (LMW) coagulation proteins in the CSF. These included FDP, plasminogen, and/or factor IX. The three remaining patients underwent lumbar puncture 4-16 days after clinical onset and none showed more than one LMW coagulation protein in the CSF. In contrast, CSF from patients studied at a later stage after subarachnoid haemorrhage (group 2) showed no abnormality.

Eight out of 11 postoperative patients had two or more LMW coagulation proteins in their CSF and had recently undergone major craniotomies for syringomyelia (6) or for intracranial haemorrhage (2). In contrast, the three patients who had undergone less traumatic procedures—ventriculography (2) or insertion of a Spitz-Holter valve (1)—had no CSF abnormality.

The seven infected patients with two or more LMW coagulation proteins in their CSF suffered from acute bacterial meningitis, while three patients without this CSF abnormality had suffered only mild viral encephalitis.

Two out of the five patients with a spinal tumour had two or more LMW coagulation proteins in their CSF and they had complete myelographic block from metastases. The three who did not have the CSF abnormality (one meningioma, one metastasis, and one angiolipoma) had only partial block. Four patients with an intracranial tumour (two chromophobe adenomas and two acoustic neuromas) had two or more LMW coagulation proteins in their CSF while eight did not, and the CSF from four of the eight (two gliomas, one craniopharyngioma, and one dermoid) was completely normal.

Patients with cerebrovascular disease had a much lower incidence of LMW coagulation protein in the CSF. The three who did suffered from intracranial haemorrhage, spinal cord infarction, and cerebral atherosclerosis respectively.

GROUP 2

None of the five patients from whom ventricular CSF was obtained at craniotomy for clipping of an aneurysm up to two months after haemorrhage (non-acute subarachnoid) had any CSF abnormality. Only nine out of the whole group of 188 patients had two or more LMW coagulation proteins in their CSF. Three, classified as 'degenerative', had peripheral neuropathies with a gross rise in CSF protein, one had an acute disc prolapse (lumbar spondylosis group), one had ischaemic myelopathy (cervical spondylosis group), and one had a long-standing Spitz-Holter valve for hydrocephalus (miscellaneous group). The two patients in whom no neurological abnormality was detected (NAD) and the one epileptic had not developed any new neurological abnormality after full investigation and 18 months' follow-up.

The presence of two or more LMW coagulation proteins in the CSF was not associated with a raised CSF red cell count except in the acute subarachnoid haemorrhage and postoperative patients. If these patients are excluded only four out of 25

Table 1 *Range of normal concentrations for CSF proteins obtained from 22 patients who proved to be clinically normal. Total protein values represent the laboratory normal range*

CSF protein	*Concentration*	*Molecular weight**
Coagulation proteins		
FDP (fragments D and E)	< 2 μg/ml†	50-83 000
Plasminogen	<20 units†	89 000
Factor IX	< 1%†	50 000
Factor V	< 1%†	290 000
Fibrinogen	nil	340 000
Total protein		
Lumbar	<0·45 g/l	Predominantly 45-90 000
Cisternal	<0·25 g/l	
Ventricular	<0·15 g/l	

*Molecular weight for coagulation proteins taken from Hardisty (1974).
†Limitation of method; no factor activity detected.

patients with a raised CSF red cell count had LMW coagulation proteins. A raised CSF total protein (predominantly of MW 45-90 000) correlated significantly with raised FDP ($r = 0·46$, $P < 0·001$) and with raised plasminogen ($r = 0·842$, $P < 0·001$) but not raised factor IX. A correlation between CSF plasminogen and total protein in various neurological disorders has previously been reported (Wu *et al.*, 1973).

The level of plasminogen activator in CSF was measured in 18 of the patients with LMW coagulation proteins in their CSF. A clear zone of lysis of the fibrin plate was observed in seven patients but there was no correlation with clinical diagnosis or the type of LMW coagulation protein present. Only one of the four patients with acute subarachnoid haemorrhage who were tested showed an increased zone of fibrin lysis; his CSF was tested within 24 hours of clinical onset compared with three, six, and 11 days for the others.

Discussion

Fibrin/fibrinogen degradation products may appear in CSF under pathological conditions even with a normal serum FDP level (Hunter *et al.*, 1974; Brueton *et al.*, 1976), but the assumption that their presence in CSF is solely a consequence of increased CSF fibrinolysis (Tovi, 1972; Hunter *et al.*, 1974) now seems unlikely. We found FDP in the CSF of 23·4% of patients undergoing diagnostic lumbar or ventricular puncture compared with 18·5% in an earlier report (Hunter *et al.*, 1974). Filtration of protein into the CSF compartment is known to be inversely related to molecular size (Schliep and Felgenhauer, 1974), and these low molecular weight FDP (fragments D and E) were present in association with other coagulation proteins of similar molecular size which are also not normally detectable in CSF.

These proteins were found in patients with in-

Table 2 *Results for patients in group 1 (loss of integrity of blood-CSF barrier) and group 2 (normally intact blood-CSF barrier)*

Diagnosis	*No. of patients studied*	*No. of patients with raised CSF level*				*Patients with two or more LMW coagulation proteins in CSF*	
		LMW coagulation proteins			*Total protein*		
		FDP	*Plasminogen*	*Factor IX*		*No.*	*%*
Group 1							
Acute subarachnoid	13	12	10	7	10	10	76·9
Postoperative	11	11	9	8	11	8	72·7
Infected	10	7	8	4	8	7	70·0
Tumour							
spinal	5	2	4	0	4	2	40·0
intracranial	12	6	4	4	7	4	33·3
Cerebrovascular disease	13	3	4	1	8	3	23·0
Total	64	41	39	24	48	34	53·1
Group 2							
Non-acute subarachnoid	5	0	0	0	—	0	0
Degenerative	21	5	5	0	8	3	14·3
NAD	27	2	2	0	4	2	7·4
Spondylosis							
lumbar	15	1	4	0	9	1	6·7
cervical	20	4	1	0	6	1	5·0
Epilepsy	19	1	3	2	4	1	5·3
Demyelination	27	1	3	0	3	0	0
Cerebral atrophy	26	2	2	0	8	0	0
Miscellaneous	28	2	6	2	8	1	3·6
Total	188	18	26	4	50	9	4·8
Total of groups 1 and 2	252	59	65	28	98	43	17·1

LMW = low molecular weight: NAD = no abnormality detected.

flammatory, vascular, and neoplastic conditions, which are known to increase the permeability of meningeal and choroidal capillaries, and the findings are consistent with protein leakage across a damaged blood-CSF barrier. Non-specific damage to this barrier is common to various neurological conditions (Schliep and Felgenhauer, 1974) and the presence of these coagulation proteins is unlikely to provide any further discriminant diagnostic function. A similar finding for CSF immunoglobulin and complement in meningitis was reported by Whittle and Greenwood (1977).

The relative contribution of increased permeability of the blood-CSF barrier to FDP, as opposed to their local generation by fibrinolysis, is likely to vary in different pathological conditions. This is analogous to the source of origin of FDP in urine in glomerulonephritis and the nephrotic syndrome (Clarkson *et al.*, 1971; Naish *et al.*, 1974; Hall *et al.*, 1975) and of FDP in peritoneal and pleural fluids (Benz, 1968; Svanberg and Åstedt, 1975; Åstedt *et al.*, 1976). The presence of raised CSF but not serum FDP is not an argument against leakage across the blood-CSF barrier. Intravascular thrombosis and clot lysis within cerebral vessels may generate a high local concentration of FDP that can leak into CSF but cannot reach, or may be diluted in, the blood of the systemic circulation.

Clinical trials of fibrinolytic inhibition in subarachnoid haemorrhage should therefore include a serial study of both FDP and plasminogen activator in CSF, particularly in the control group, since this may be of prognostic value. Our limited data suggest that these proteins usually disappear from the CSF within three to four days and their persistence (Tovi *et al.*, 1973; Smith and Upchurch, 1973; Brueton *et al.*, 1976) may indicate a continuing local lesion. The selection of patients for antifibrinolytic therapy, however, should not be based on the assumption that FDP in the CSF necessarily reflects increased fibrinolytic activity in the subarachnoid space.

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