Rheological and fibrinolytic findings in multiple sclerosis

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SUMMARY Blood viscosity indices, fibrinolytic activity and other serum proteins related to haemocoagulation have been studied in 36 patients with multiple sclerosis. The whole blood viscosity was found to be increased in multiple sclerosis. The increase was caused by a decrease in erythrocyte deformability since plasma viscosity and haematocrit were normal. Plasminogen, fibrinogen and α_1 antitrypsin levels were found to be lower than normal. Such alterations were not observed in a group of patients with other non-immunological neurological diseases. In the latter group some coagulation indices were even higher than normal. The higher mean age of the pathological controls could explain the observed levels. The abnormalities observed in multiple sclerosis patients are considered to be a consequence of a non-specific activation of the coagulative system in a chronic immunological disease.

The elevated frequency of an IgG increase in multiple sclerosis (MS) cerebrospinal fluid (CSF) has stimulated several workers to careful estimations of IgG in the serum of MS patients. Little attention has been paid to other (non Ig) serum proteins.¹ The most constant data are concerned with complement activity and complement factors² and with the coagulation system. Menon *et al* found an increase of fibrinolytic activity in MS serum, which suggested the possibility of an alteration of the coagulative system.³ Such a possibility also was evaluated by Caspary *et al*,⁴ who reported an increase of the platelet stickiness in MS. The increased platelet adhesiveness also was found to be correlated to disease activity and to adrenocorticotrophic therapy.^{5, 6} The alteration of the coagulative system has even been considered to be directly or indirectly related to plaque pathogenesis.⁷ We report the results obtained in a study of the blood viscosity indices, fibrinolytic activity and other serum proteins concerned in coagulation in 36 patients with MS. The results obtained were compared with a normal group and a group of patients with various non-immunological neurological diseases.

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Material and methods

Patients and controls

Thirty-six patients affected by "probable MS"⁸ were evaluated. Twelve were male and 25 were female, with a mean age of 32 years (range 19-66). All these patients were in hospital and were chronic cases with symptoms of a recent relapse or gradual worsening. All received corticosteroids or ACTH during the six months preceding evaluation. Twelve inpatients (two male, 10 female) were taken as pathological controls. They suffered from parkinsonism (2), epilepsy (2), presenile dementia (2), cerebrovascular disease (3), amyotrophic lateral sclerosis (1) sciatic pain (2). The mean age of this group was 54 years (range 45-64). Thirty normal subjects (10 male and 20 female) with a mean age of 34 years (range 25-45) were taken as normal controls.

Measurements were made of whole blood and plasma viscosity, haematocrit (Ht), fibrinogen, plasminogen, euglobulin lysis time (ELT), antithrombin III (AT III), α_1 antitrypsin (α_1 -AT).

Whole blood and plasma viscosity were evaluated in EDTA anticoagulated blood (1 mg/ml) in a Wells Brookfield LVT cone-plate viscometer (Brookfield Labs, Stoughton, Mass, USA) according to the method proposed by Wells *et al*.⁹ Aliquots of 2 ml of whole blood and plasma were used in each instance; the test was carried out in duplicate and the average value was used. Blood and plasma viscosity were evaluated at 37°C. This temperature was constantly maintained by means of a thermostatic pump (Colora, model N) supplied by Brookfield Labs. Readings were carried out in every instance after a 30 s stabilisation period at speed 60 which corresponds to

230 s⁻¹ shear rate. Relative viscosity was calculated by the ratio between whole blood viscosity and plasma viscosity as obtained at 230 s⁻¹. For the other tests blood was drawn into plastic syringes containing 3.8% sodium citrate (1 ml of anticoagulant for 9 ml of blood). The blood was then placed in plastic tubes which were centrifuged at 1 000 g for 15 min. After centrifugation the plasma was separated and then frozen at -20°C. All tests were done within 10 days of sample collection.

Haematocrit values were carried out according to standard procedures. For the assessment of plasma fibrinolytic activity we used the Euglobulin Lysis Time according to the method proposed by Clifton *et al.*¹⁰

Fibrinogen, plasminogen, AT III and α 1-AT were evaluated using single radial immunodiffusion test (M-Partigen, Behringwerke Labs) according to Mancini's method.¹¹ For statistical evaluation we used the "t" and the Fisher's exact test.

Table 1 Mean SD and statistical variance analysis of the rheological indices in the normal group compared to the MS group

	Mean	SD	F	p
Whole blood viscosity in cps				
MS	4.59	0.51	—	—
Normals	4.37	0.28	3.97	< 0.05
Plasma viscosity in cps				
MS	1.51	0.27	—	—
Normals	1.51	0.10	0.01	NS
Relative viscosity				
MS	3.09	0.27	—	—
Normals	2.85	0.02	5.87	< 0.05
Haematocrit %				
MS	42.08	3.68	—	—
Normals	43.46	2.78	2.86	NS

Table 2 Mean SD and statistical variance analysis of the fibrinolytic and serum proteins indices in the normal and in the pathological controls compared to the MS group

	Mean	SD	F	p
Fibrinolysis in hours				
MS	19.08	8.72	—	—
Normals	21.46	5.21	1.72	NS
Fibrinogen in mg %				
MS	302.94	98.30	—	—
Normals	342.00	49.64	3.89	< 0.05
Pathological controls	431.66	53.96	18.67	< 0.005
Plasminogen in mg %				
MS	9.80	2.67	—	—
Normals	11.57	1.75	12.45	< 0.005
Pathological controls	16.61	5.31	39.58	< 0.005
AT III in mg %				
MS	28.91	7.91	—	—
Normals	28.86	3.66	0.00	NS
Pathological controls	34.81	13.35	3.49	< 0.005
α 1-AT in mg %				
MS	135.38	69.83	—	—
Normals	217.83	40.16	32.64	< 0.005
Pathological controls	206.75	70.53	4.29	< 0.05

Table 3 Mean SD and statistical variance analysis of the coagulation indices in the normal control group compared to the pathological control group

	Mean	SD	F	p
Plasminogen in mg %				
Normals	11.57	1.75	—	—
Pathological controls	16.61	5.31	21.80	< 0.005
Fibrinogen in mg %				
Normals	342.00	49.64	—	—
Pathological controls	431.66	53.96	25.29	< 0.005
AT III in mg %				
Normals	28.86	3.66	—	—
Pathological controls	34.81	13.35	5.21	< 0.05
α -1AT in mg %				
Normals	217.83	40.16	—	—
Pathological controls	206.75	70.53	0.11	NS

Table 4 Correlation coefficient r, t and p obtained by comparing some of the tests used

Correlation	MS no of cases	r	t	p
Whole blood viscosity/haematocrit	36	+0.79	7.47	< 0.001
Plasma viscosity/fibrinogen	36	-0.38	2.43	< 0.02
Plasma viscosity/plasma globulins	35	+0.36	2.21	< 0.05
Relative viscosity/haematocrit	36	+0.43	2.84	< 0.01
Fibrinogen/fibrinolysis	36	+0.03	0.15	NS
Plasminogen/fibrinolysis	36	+0.13	0.78	NS
Plasminogen/fibrinogen	36	+0.36	2.25	< 0.025

Fig 1 Correlation between whole blood viscosity (ordinate) and haematocrit (abscissa) in the MS patient group.

Fig 2 Correlation between relative viscosity (ordinate) and haematocrit (abscissa) in the patients with MS.

Fig 3 Plasma viscosity and plasma globulins correlation in the MS patient group.

Results

The results are summarised in tables 1–4 and in figs 1–3. The difference of the means between the MS and the normal group was found to be statistically significant for whole blood viscosity, relative viscosity, fibrinogen, plasminogen and α 1-AT. An increase was found in the pathological control group compared to the MS group for the following tests: fibrinogen, plasminogen and α 1-AT levels. An increase in the pathological control group compared to the normal group was seen for fibrinogen, plasminogen and AT III levels.

There were some correlations between the tests investigated. The whole blood viscosity and the relative viscosity correlated with the haematocrit values (figs 1 and 2). A negative correlation was seen between plasma viscosity and fibrinogen values ($r = -0.38$; $t = 2.43$; $p < 0.025$). Statistically sig-

nificant correlations were also found between fibrinogen and plasminogen levels ($r = +0.36$; $t = 2.35$; $p < 0.05$) and between plasma viscosity and globulin levels (fig 3).

Discussion

Our data confirm and amplify the abnormalities of the coagulative system reported in MS. Increased whole blood viscosity in MS has been reported,¹² as well as increased fibrinolytic activity.³ The latter parameter was increased slightly in our cases, but this was not a significant difference from the normal controls. This may be due to differences in case selection. Our group was composed of inpatients with recent deterioration, on corticosteroid or adrenocorticotrophic therapy. Menon's patients were ambulant and not receiving treatment. Whole blood viscosity is known to be strongly influenced by the Ht values, and this appears to be true also for MS cases. However, while the Ht values were normal in the MS group, the blood viscosity was increased (that is, the viscosity increase is not only due to the Ht changes). Since fibrinogen is decreased and globulins are not particularly increased, such an increase of whole blood viscosity is probably due to an increase of relative viscosity, that is to decreased erythrocyte deformability.¹³ That this is so was demonstrated by the observation that relative viscosity was elevated in the patients as compared to controls. The alterations of the coagulative system in MS was confirmed also by the reduced levels of plasminogen, fibrinogen and α 1-AT.

The observed alterations of the coagulative system may be related to the basic pathological process in MS. The alterations observed in MS are concerned mainly with the immunological system. An increase of CSF IgG levels, often with oligoclonal bands, is extremely common.¹⁴ In the serum, reduction of complement factors,² probable presence of immuno-complexes,¹⁵ and defective antibody production also have been described.¹⁶ In other immunological diseases characterised by the presence of antigen-antibody complexes, the coagulative system also is activated by an indirect mechanism. In SLE, for example, slight disseminated intravascular coagulation has been described.¹⁷ Related to this may be the observation that in MS there is increased platelet stickiness and platelet release.⁴

It is quite possible that in MS the immunological abnormalities would activate the coagulation cascade with fibrinogen consumption. Furthermore, the formation of fibrin will increase the fibrinolytic activity. As a consequence, as in classic disseminated intravascular coagulation, plasminogen also will be

activated to plasmin with reduction of the plasminogen level. The plasmin, in turn, will react with the α 1-AT which, with the α 2-macroglobulin, is the most important antagonist and inhibitor of the plasmin.¹⁸

The coagulative indices in MS and other pathological controls were compound. In the latter group we observed an increase of plasminogen, fibrinogen and α 1-AT compared to both MS and normal groups. Such divergent behaviour of some coagulation factors in MS and other non-immunological neurological diseases may be explained by the fact that the mean age of the pathological control group was substantially higher than the mean age of the MS and normal groups. It is known that with age there is an increase of several coagulation factors (fibrinogen and plasminogen, for instance).¹⁹ Furthermore, reduced mobility had probably affected both pathological groups in similar manner. Therefore, stasis and the prethrombotic state appeared to be of slight importance. In this regard it is important to note that no patient of the other pathological groups has ever shown clinical symptoms of thrombophlebitis or thrombotic state during the study.

Finally, we want to stress that all the observed abnormalities of the coagulation system can be explained on the basis of a chronic immunological disease. Therefore, such alterations are not necessarily involved in a direct way in the genesis of demyelination in MS plaques.

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