J. Neurol. Neurosurg. Psychiat., 1966, **29**, 187

Platelet stickiness in multiple sclerosis

J. H. D. MILLAR, J. D. MERRETT, AND ANNE M. DALBY

From the Department of Neurology, Royal Victoria Hospital, Belfast, and the Department of Medical Statistics, the Queen's University of Belfast

Nathanson and Savitsky (1952) were the first to study platelet adhesiveness in patients suffering from multiple sclerosis (60 in all). Increased adhesiveness was found in the groups of patients with acute exacerbations or a fluctuating course: the average 'adhesive index' for the stationary group was within the normal range. More recently two preliminary communications were published on the same subject in the *Lancet*: Caspary, Prineas, Miller, and Field (1965) found increased platelet adhesiveness in 43 patients suffering from active multiple sclerosis. Wright, Thompson, and Zilkha (1965) also found platelet adhesiveness to be increased in 24 patients suffering from acute severe multiple sclerosis. However, 12 patients were receiving or had recently received corticotrophin therapy. Sharp (1965) found no increased adhesiveness when the disease was quiescent and the proportion of cases showing abnormal adhesiveness increased with the activity of the disease.

METHOD

The method used measures the adhesion of platelets to a glass surface. A venous sample of blood is taken and anticoagulated with heparin, and a platelet count is done (initial count). A sample of the blood is then placed in a non-siliconized glass bulb which is slowly rotated for 20 minutes and a further platelet count is done (final count). The final count is expressed as a percentage of the initial count and this gives the platelet stickiness; the lower this figure the higher the proportion of platelets which have adhered to the glass surface and therefore the more sticky the platelets. The platelet counts shown in the tables are the number of platelets counted in a field $\frac{2}{3}$ sq. mm.

MATERIAL

Fifty patients, 17 men and 33 women, suffering from multiple sclerosis, were studied. The majority were outpatients; none was bedridden or suffering from urinary infection or vascular disease. The only treatment they had received during the previous year was aneurin. The patients were divided into active and inactive groups. The active group consisted of 16 patients in whom the disease appeared to be active or in relapse and the inactive group consisted of 34 patients in whom the disease process was stationary or in remission. Thirty-three apparently healthy persons, some of whom were medical students or nurses, comprised the control group.

METHOD OF ANALYSIS

The index of platelet stickiness, *i.e.*, a subject's final platelet count as a percentage of his initial count, being a ratio of two variables, does not lend itself to valid statistical analyses, such as t tests and analysis of variance, designed to test the significance of differences between groups in terms of 'average' stickiness. Consequently, in what follows, comparisons between groups have been made in terms of average final counts 'adjusted' to a common level of initial platelet count. Moreover, as there is little evidence about the possible influence of age and sex on platelet stickiness we have treated each sex separately, giving three groups of male patients and three groups of female patients, and we have examined the possible effect of age on the comparisons between adjusted group means. The method of analysis is essentially that of the analysis of covariance (Quenouille, 1952).

RESULTS

Table I gives the average age and average initial and final counts in each of the six groups. It also includes the average index of platelet stickiness estimated in the usual way, *i.e.*, if y is the final count and x the initial count the average index is the sum of, say n values, of $100y/x$ divided by n. These indices suggest that platelet stickiness is increased in active cases of multiple sclerosis compared with either control subjects or inactive cases of multiple sclerosis; the differences between the groups are more striking for females than for males.

For each of the six groups we derived a regression equation of average predicted final count on initial count and age but in only one of the groups did age make any significant (at the probability level of 5% used throughout) contribution to the prediction of average final count. Even in this case the contribution was small, namely, an average decrease of 1·6 in predicted final count for an increase of one year of age with initial count held constant. Consequently age was ignored and six new regression equations of

TABLE I

AVERAGE OF AGE, INITIAL PLATELET COUNT, AND FINAL PLATELET COUNT BY SEX AND GROUP

Group	*No. in Group*	*Average Age at Test (yr.)*	*Average Initial Platelet Count (see text)*	*Average Final Platelet Count (see text)*	*Average % (see text)*
Males					
Control	16	31·50 ± 3·69[1]	218·19 ± 16·68	160·88 ± 12·07	73·6
Active multiple sclerosis	7	35·43 ± 4·41	209·57 ± 40·18	137·86 ± 26·71	65·6
Inactive multiple sclerosis	10	38·50 ± 2·85	242·30 ± 24·33	174·10 ± 18·44	71·3
Females					
Control	17	29·53 ± 2·21	216·24 ± 14·83	158·46 ± 12·56	71·9
Active multiple sclerosis	9	36·33 ± 2·53	218·00 ± 21·51	120·22 ± 14·03	58·7
Inactive multiple sclerosis	24	38·79 ± 2·07	264·67 ± 20·47	187·46 ± 16·92	70·1

[1]Standard errors follow the ± sign.

TABLE II

ANALYSIS OF COVARIANCE OF FINAL PLATELET COUNT (Y) ON INITIAL PLATELET COUNT (X) BETWEEN GROUPS

Source of Variation	*Sums of Squares*	*Degrees of Freedom*	*Mean Square*	*Variance Ratio (F)*	*Probability (P)*
Males					
Difference between group regressions	247·93	2	123·96	<1	>0·20
Deviations from individual group regressions	5,210·03	27	192·96		
Deviations from average regression within groups	5,457·96	29	188·21		
Between adjusted group means	1,431·03	2	715·51	3·80	0·02-0·05
Total	6,888·99	31			
Females					
Difference between group regressions	1,392·61	2	696·30	<1	<0·20
Deviations from individual group regressions	31,215·15	44	709·44		
Deviations from average regression within groups	32,607·76	46	708·86		
Between adjusted group means	9,307·88	2	4,653·94	6·57	0·001-0·01
Total	41,915·64	48			

average predicted final count on initial count were derived. These took the form of

$$Y = a + bx$$

where Y = average predicted final count, x = observed initial count, and a and b are constants. Within each sex there was no evidence (see Table II) of any significant differences between the three group regressions, *i.e.*, between the b values, so that the relevant data were pooled within each sex to derive an average regression (b) within groups. As Table II shows, completion of the covariance analysis revealed significant differences for each sex between the group means of the final platelet counts after they had been adjusted to a common initial platelet count. The adjusted means and the size and nature of the differences between them are shown in Table III. The main conclusions from this table are that the adjusted mean final counts were significantly less among patients with active multiple sclerosis than among either patients with inactive multiple sclerosis or control subjects. These results indicate for each sex separately that, on average, the platelet stickiness of patients with active multiple sclerosis is signifi-

TABLE IIIa

AVERAGE ADJUSTED FINAL PLATELET COUNT BY SEX AND GROUP

Group	*Sex*	
	Male	*Female*
Control	164·7	176·4
Active multiple sclerosis	147·6	136·8
Inactive multiple sclerosis	161·2	168·6

TABLE IIIb

COMPARISON OF AVERAGE ADJUSTED FINAL PLATELET COUNTS BETWEEN GROUPS BY SEX

Sex	*Difference*		
	Control—Active Multiple Sclerosis	*Control—Inactive Multiple Sclerosis*	*Inactive Multiple Sclerosis—Active Multiple Sclerosis*
Male	17·1[1] ± 6·2	3·5 ± 5·6	13·6[1] ± 6·8
Female	39·6[1] ± 11·0	7·8 ± 8·7	31·8[1] ± 10·6

[1]Significant at $P < 0.05$. Standard errors of difference follow the ± sign.

cantly increased compared with either control subjects or patients with inactive multiple sclerosis. Furthermore the average stickiness is similar in these latter two groups.

DISCUSSION

Millar and Dalby (1965) found increased platelet stickiness in patients suffering from cerebrovascular disease but no significant difference was found between the three groups tested—acute cerebral thrombosis, chronic cerebrovascular disease, and transient cerebral ischaemic attacks—the last group being tested between attacks. They suggested that some factor in the blood made the platelets more sticky, predisposing to cerebrovascular disease. The fact that patients suffering from inactive multiple sclerosis showed normal platelet sitckiness suggests that the increased stickiness associated with the active disease process is possibly secondary, the result of a product of demyelination liberated into the blood.

Baker, Sanders, Thompson, and Zilkha (1965) found that the total serum linoleate level in patients suffering from active multiple sclerosis was significantly lowered. Wright *et al.* (1965) suggested that this lowered linolenic-acid level might be expected to increase platelet adhesiveness, as Kerr, Pirrie, MacAulay, and Bronte-Stewart (1965) had found that only linoleate and linolenate of the free fatty acids they tested did not cause platelet aggregation, and lecithin and linolenate inhibited platelet aggregation caused by saturated fatty acids. However, Borchgrevink, Berg, Skaga, Skjaeggestad, and Stormorken (1965) reported that linolenic acid by mouth had no effect on platelet adhesiveness in men suffering from coronary heart disease. Caspary *et al.* (1965) suggested that a heavy fat meal increased platelet adhesiveness in patients suffering from multiple sclerosis. We found no difference in platelet stickiness two hours after both a low-fat and a high-fat breakfast in 11 patients suffering from multiple sclerosis and 10 control subjects.

SUMMARY

Platelet stickiness is increased in patients suffering from active multiple sclerosis compared with patients suffering from inactive multiple sclerosis and normal controls.

We wish to thank the Northern Ireland Hospitals Authority for a grant to defer expenses.

REFERENCES

Baker, R. W. R., Sanders, H., Thompson, R. H. S., and Zilkha, K. J. (1965). Serum cholesterol lineolate levels in multiple sclerosis. *J. Neurol. Neurosurg. Psychiat.*, **28**, 212-217.

Borchgrevink, C. F., Berg, K. J., Skaga, E., Skjaeggestad, Ø., and Stormorken, H. (1965). Effect of linseed oil on platelet adhesiveness and bleeding-time in patients with coronary heart disease. *Lancet*, **2**, 980-982.

Caspary, E. A., Prineas, J., Miller, H., and Field, E. J. (1965). Platelet stickiness in multiple sclerosis. *Ibid.*, **2**, 1108-1109.

Kerr, J. W., Pirrie, R., MacAulay, I., and Bronte-Stewart, B. (1965). Platelet-aggregation by phospholipids and free fatty acids. *Ibid.*, **1**, 1296-1299.

Millar, J. H. D., and Dalby, A. M. (1965). Platelet stickiness in cerebrovascular disease. *Proceedings 8th International Congress of Neurology, Vienna*, 1965, Vol. 4, pp. 483-487.

Nathanson, M., and Savitsky, J. P. (1952). Platelet adhesive index studies in multiple sclerosis and other neurologic disorders. *Bull. N.Y. Acad. Med.*, **28**, 462-468.

Quenouille, M. H. (1952). *Associated Measurements*. Butterworth, London.

Sharp, A. A. (1965). Platelets in multiple sclerosis. *Lancet*, **2**, 1296-1297.

Wright, H. P., Thompson, R. H. S., and Zilkha, K. J. (1965). Platelet adhesiveness in multiple sclerosis. *Ibid.*, **2**, 1109-1110.