whereas, with four doubtful exceptions, the test was never positive in patients in whom the antibody had not been recently acquired. In short, in this population infectious mononucleosis accompanied by a positive Paul-Bunnell test was an entity associated with the development of EB virus antibody. On the other hand, cases resembling infectious mononucleosis but with a negative Paul-Bunnell test may be caused by a variety of agents.

A differing virological origin for cases of infectious mononucleosis with a positive Paul-Bunnell test and for cases with very similar clinical and haematological features but in whom the Paul-Bunnell test is negative goes far to explain the differences that have been reported between such positive and negative cases. These differences include incubation period, the age groups mainly affected, infectivity, and the occurrence of outbreaks (Hobson *et al.*, 1958; Hoagland, 1967; Finch, 1969).

Our thanks are due to Sir Austin Bradford Hill, who read the draft and made many helpful comments. We also wish to record our appreciation of the co-operation of the students, and of the help

given by nursing and clerical staff of the university and college health centres.

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Platelet Defect of Infectious Mononucleosis

ROBERT CLANCY, ELIZABETH JENKINS, BARRY FIRKIN

British Medical Journal, 1971, 4, 646-648

Summary

Platelet function has been studied in 16 patients with uncomplicated infectious mononucleosis. Some abnormality of platelet aggregation and/or release of platelet factor III or platelet factor IV was found in all patients. Six patients with platelet defects were retested after three to four months and were found to have normal platelet function and appreciably higher platelet counts. Abnormal platelet function may reflect a platelet defect which predisposes to premature platelet destruction. Recent viral illness should be excluded before attributing abnormal platelet function to other factors or to a congenital disorder.

Introduction

Infectious mononucleosis, as well as a number of other viral diseases, can be associated with thrombocytopenia of all grades (Ackroyd, 1949; Hudson *et al.*, 1956). Asymptomatic thrombocytopenia is common (Carter, 1965; Cantow and Kostinas, 1966), but thrombocytopenic purpura occurs only as a rare complication (Sharp, 1969). The pathogenesis of virus-associated thrombocytopenia is unknown but may involve several mechanisms.

This study shows that abnormal platelet function is common in uncomplicated infectious mononucleosis, and it is suggested that these abnormalities reflect platelet damage which may predispose to premature platelet destruction.

Patients and Methods

Sixteen patients (10 females and 6 males) were studied in the second or third week of illness. Their ages ranged from 16 to 35 years, except for one woman who was 58. Most of them had been admitted to hospital because of the severity of symptoms, thus providing a selection bias to this series. Fifteen presented with a typical "pharyngeal" onset of sore throat, fever, malaise, and lymphadenopathy. One (Case 10) presented with hepatic involvement, and several others had abnormal liver function tests. Splenomegaly was present in six. All patients had a peripheral blood picture consistent with infectious mononucleosis, the most striking feature being an atypical lymphocytosis. The Paul-Bunnell test for heterophil antibody was positive in all patients after serum was absorbed with guinea-pig kidney. No patient had purpura. The Hess test was negative in all cases and an Ivy bleeding time was normal in the eight tested. Patients had not taken aspirin within four days of study, though some had ingested aspirin earlier in their illness. Six who had platelet defects were studied three to four months after the onset of illness; all were well at that time and had negative Paul-Bunnell tests and normal blood films, except for occasional atypical lymphocytes.

PLATELET INVESTIGATIONS

Platelet counts were performed by the method of Brecher and Cronkite (1950). Platelet factor III availability was determined by incubation of platelet-rich plasma with kaolin at 37°C, with assay of platelet coagulant activity released at 1, 5, 10, and 20 minutes, and expressed as reduction in the Stypven-calcium time after 20 minutes' incubation (Spaet and Cintron, 1965; Hardisty and Hutton, 1965). Platelet factor IV (anti-heparin factor) was determined by prolongation of the thrombin time in the presence of heparin by platelet-rich plasma (Niewiarowski and Thomas, 1969).

Platelet aggregation was studied by a modification of the method of Born (1962) and O'Brien (1962). Aggregating agents and their final concentrations were: adenosine diphosphate

Monash University Department of Medicine, Alfred Hospital, Melbourne, Australia

ROBERT CLANCY, B.Sc., M.R.A.C.P., N.H. & M.R.C. Research Fellow
ELIZABETH JENKINS, Technical Officer
BARRY FIRKIN, B.Sc., F.R.A.C.P., Professor of Medicine

(ADP) 5×10^{-6} M, adrenaline 5×10^{-6} M, thrombin 0.1 unit/ml, and ristocetin 1 mg/ml. (Ristocetin is an antibiotic withdrawn from use because of the high incidence of thrombocytopenia, and found to be a useful aggregating agent in assessing platelet function (Howard and Firkin, 1971).) Aggregation was measured as the maximal change in optical density caused by aggregate formation, expressed as a percentage of the optical density difference between platelet-rich and platelet-poor plasma. Tests of platelet aggregation were performed at a platelet concentration of $280,000/\text{mm}^3$ and at a temperature of 37°C . Platelet-poor plasma from five patients with platelet defects was tested for its ability to inhibit aggregation of normal platelet-rich plasma with ADP or ristocetin. Normal platelet-rich plasma (1.5 ml) was incubated with 0.5 ml of patient's or normal platelet-poor plasma for 30 minutes at 37°C . ADP or ristocetin was then added and the optical density change measured, as above.

Antiplatelet antibodies were assessed by antibody-kaolin-induced platelet factor III release. Details of this technique are to be published (Clancy *et al.*, 1971). In brief, the globulin fraction from a patient's serum is incubated with normal platelet-rich plasma (platelet count $400,000/\text{mm}^3$) at 37°C for 30 minutes. The one-minute Stypven-calcium clotting time is determined after the addition of kaolin and compared with clotting times obtained with control globulin fractions. A test is considered to be positive for antiplatelet antibody when the clotting time is reduced by more than four seconds, compared with controls, on at least two separate occasions. This test is positive in 65% of adult patients with idiopathic thrombocytopenic purpura and is negative in non-immunological thrombocytopenia. Fibrinogen degradation products were determined by haemagglutination inhibition (Merskey *et al.*, 1969).

Results

Platelet counts were normal in all patients. Fourteen had abnormal platelet aggregation; 11 had reduced aggregation with ADP and 13 with ristocetin (see Table). Impaired aggregation with adrenaline and thrombin is more difficult to assess because of the wide normal range and the finding that some normals do not aggregate with adrenaline. In our laboratory 15% of normals did not aggregate with adrenaline, and these have been excluded in calculating the normal range. This figure is similar to that found by other workers (Logan *et al.*, 1971). However, only four patients aggregated within this range with adrenaline, indicating a probable reduced response to this agent. Variation in platelet aggregation with thrombin was also considerable but in 20 control subjects all had greater than 20% aggregation. As only five patients had greater than

20% aggregation with thrombin, aggregation was probably also impaired with this agent.

All eight patients tested had impaired platelet factor IV release, and four had reduced platelet factor III availability. Convalescent platelet function was normal in the six patients tested. The mean platelet count of these patients in the acute stage ($214,000/\text{mm}^3$) was less than that in convalescence ($267,000/\text{mm}^3$) ($P < 0.05$). Platelet-poor plasma from five patients, all of whom had reduced aggregation with ADP and ristocetin, did not inhibit aggregation of normal platelet-rich plasma with either of these agents when compared with the effect of normal platelet-poor plasma. Antiplatelet antibody was not detected in any patient, and fibrinogen degradation products were either in the normal range or slightly raised.

Discussion

Infectious mononucleosis is a good model for the investigation of platelet-virus interaction, as it presents a definable population of patients in which a spectrum of quantitative platelet abnormalities can be found. This study shows that reduced platelet aggregation and impaired release of platelet factors III and IV are fairly common in patients with infectious mononucleosis which is uncomplicated but severe enough to require admission to hospital. In six studied in convalescence the platelet function was normal and the platelet count was significantly higher than it had been in the acute phase.

The pathogenesis of the platelet defect could not be determined from these studies. Known depressants of platelet function, such as antiplatelet antibody (Clancy *et al.*, 1971) and fibrin degradation products (Merskey *et al.*, 1969) were not detected. Incubation of normal platelet-rich plasma with platelet-poor plasma from patients with infectious mononucleosis and abnormal platelet function did not induce abnormal platelet function. Possible mechanisms would include virus uptake and platelet damage, as suggested by ultrastructural studies (Danon *et al.*, 1959), or altered cell behaviour due to a virus-directed membrane-associated protein (Roizman and Spear, 1971). Alternatively, an immune response stimulated by the virus could cause platelet damage as a result of antibody directed against a virus-platelet complex, or from adsorption of immune complexes to the platelet membrane.

Immune complexes aggregate platelets (Luscher, 1970) and platelet aggregation has been used, in the presence of antigen, as a test for antibody in viral infections (Myllyla *et al.*, 1969). Thus it is possible that abnormal platelet function reflects a defect induced by non-specific immune complexes and that this defect determines premature platelet destruction and thrombocytopenia. Finally, aspirin ingestion may cause

Platelet Function in Patients with Infectious Mononucleosis

Case No.	Stage of Disease*	Platelet Count (mm^3)	Platelet Factor III Release (Sec.)	Platelet Factor IV Release (Sec.)	Platelet Aggregation (%)			
					ADP	Ristocetin	Adrenaline	Thrombin
1	Acute	170,000	9	N.D.	20	17	15	15
	Conval.	255,000	12	2	70	65	50	50
2	Acute	150,000	18	N.D.	9	11	2	5
	Conval.	250,000	17	0	57	70	50	25
3	Acute	210,000	N.D.	N.D.	25	35	20	17
	Conval.	225,000	12	2	63	82	73	50
4	Acute	210,000	2	18	65	5	15	5
	Conval.	330,000	10	2	50	75	17	45
5	Acute	200,000	11	N.D.	40	34	38	38
	Conval.	220,000	20	1	50	55	10	10
6	Acute	345,000	8	16	40	30	45	45
	Conval.	320,000	9	6	80	90	80	70
7	Acute	180,000	18	12	23	20	30	2
8	Acute	300,000	6	58	25	5	8	16
9	Acute	370,000	7	16	26	25	5	25
10	Acute	200,000	0	15	30	15	10	10
11	Acute	175,000	3	N.D.	33	39	13	16
12	Acute	220,000	0	54	50	10	15	0
13	Acute	200,000	N.D.	N.D.	66	0	6	0
14	Acute	400,000	N.D.	N.D.	73	73	10	73
15	Acute	340,000	6	20	80	12	18	80
16	Acute	150,000	9	N.D.	20	8	8	8
Normal		150,000-450,000	>6	<10	45-95	35-95	20-95	20-95

*Acute = Studies in acute phase. Conval. = Studies three to four months after acute illness. N.D. = Not done.

abnormal platelet function. This is unlikely to be a significant factor in the series because of the different pattern of abnormality (Marcus, 1969) and the presence of abnormal function in patients who had not taken aspirin.

The higher platelet count and normal platelet function found in six patients studied in convalescence suggests that abnormal platelet function may reflect a platelet lesion predisposing to platelet destruction. Megakaryocyte hyperplasia in bone marrow smears from patients with uncomplicated infectious mononucleosis and mild thrombocytopenia (Boyd and Reid, 1968) supports the concept that platelet destruction determines thrombocytopenia in infectious mononucleosis. As thrombocytopenia occurs in about half of the patients with infectious mononucleosis at some stage of disease (Carter, 1965; Cantow and Kostinas, 1966), increased platelet removal would occur in most patients. That intracorporeal lesions can determine premature platelet destruction has been well established (Murphy *et al.*, 1969).

The relationship of abnormal platelet function to the rare complication of thrombocytopenic purpura is less clear. In several patients studied with thrombocytopenia complicating specific viral infections, very short isologous platelet survival indicated an antiplatelet factor. One of these patients had transient abnormalities of platelet function on recovery (Clancy, 1971), suggesting a possible link with the more common asymptomatic form of thrombocytopenia. However, because of the rarity of thrombocytopenic purpura, other mechanisms could be involved in determining very low platelet counts. A second consequence of abnormal platelet function is impaired haemostasis, and such defects may account for the purpura occasionally seen in patients with only mild to moderate thrombocytopenia in infectious mononucleosis (Schumacher and Barcay, 1962).

The role of abnormal platelet function in determining the outcome of virus-platelet interaction in other infections is not known. It is of interest, however, that a patient with rubella had similar platelet abnormalities (Clancy, 1971), suggesting that abnormal platelet function may occur in a number of virus infections. It should be stressed, therefore, that with the widespread use of tests of platelet function,

exclusion of recent viral infection should be made before abnormalities are attributed to other factors or congenital disorders. Finally, relapse in idiopathic thrombocytopenic purpura is often precipitated by virus infection. In this condition platelet abnormalities may persist in remission (Clancy *et al.*, 1971) and an added defect induced by virus infection may lead to an exacerbation of purpura.

This study was supported by the National Health and Medical Research Council. We would like to thank Dr. J. Forbes, medical superintendent of Fairfield Hospital, Melbourne, Australia, for permission to study some of the patients included in this report.

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Oestrogen/Progestogen Oral Contraception and Blood Clotting: A Long-term Follow-up

L. POLLER, JEAN M. THOMSON, WENDY THOMAS

British Medical Journal, 1971, **4**, 648-650

Summary

In a long-term study of women taking the combined oestrogen/progestogen oral contraceptives Ortho-Novin and Norinyl-1 the follow-up has been continued for three years and four years respectively.

Acceleration of both "intrinsic" and "extrinsic" clotting tests and of specific factor VII and X assays, reported previously at the nine-month stage, persisted throughout the period of study. Acceleration of Chandler's tube platelet aggregation and hypercoagulable thromb-

elastographic patterns were recorded. There was, however, no evidence of a cumulative effect after the first nine months.

Introduction

We have previously reported that blood clotting tests, including specific factor VII and X assays, became accelerated in women taking conventional oestrogen/progestogen oral contraceptives (Thomson and Poller, 1965; Poller and Thomson, 1966). These changes became significant from the third cycle of administration. When two oral contraceptives of varying hormone content—that is, Norinyl-1 and Ortho-Novin—were compared it seemed that the changes in clotting factor response were similar during the first nine months covered by our report (Poller *et al.*, 1968). We stated, however, that longer study was required to eliminate the possibility of gradual cumulative effects.

The present study is a follow-up of the same two groups of

Haematology Department, University Hospital of South Manchester, Withington Hospital, Manchester M20 8LR

L. POLLER, M.D., M.R.C.PATH., Consultant Haematologist
 JEAN M. THOMSON, F.I.M.L.T., Chief Research Assistant
 WENDY THOMAS, A.I.M.L.T., Senior Technician