

Acute and chronic biological false positive reactors to serological tests for syphilis

ABO blood groups and other investigations

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That biological false positive (BFP) reactions to serological tests for syphilis may sometimes have a genetic basis has been noted by Kostant (1972), who cited examples of such reactions in one family for two and in another for three generations.

A possible method of indicating an hereditary bias towards disease is through a study of ABO blood groups. According to Mollison (1967), the first well-established relationship between blood groups and disease was that between blood Group A and carcinoma of the stomach. Pernicious anaemia also occurs more frequently in Group A subjects than in Group O.

Amongst other associations listed by Passmore and Robson (1970) are Group O with duodenal ulcer (established), Group O with gastric ulcer and stomal ulcer after gastroenterostomy (possible), and Group A with tumours of the salivary glands (possible). Other possible associations involving Group O were rheumatic fever and infection with influenza virus A₂.

Although there are some data concerning ABO and rhesus blood groups amongst patients attending venereal disease clinics (*e.g.* Schofield, 1966), there is relatively little information concerning such blood groups in patients with BFP reactions. Morrison (1966), investigating a group of sixty pregnant women with acute BFP reactions, could find no correlation between such reactions and blood group but suggested a need for a similar study concerning chronic BFP reactions.

For these reasons it was felt worth while to make a broad study of acute and chronic false positive reactions including ABO blood groups.

Methods

A proforma was sent to venereal disease clinics in the United Kingdom for recording false positive serum tests for syphilis, inviting returns of ABO blood groups of acute and chronic reactors and the results of other pathological tests.

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False positive reactors were defined as patients in whom one or more reagin tests (*e.g.* Wasserman, VDRL, Kahn, Price's precipitation) had been repeatedly positive but in whom tests for anti-treponemal antibody (*e.g.* FTA and/or TPI test—usually performed at a Reference Laboratory) were negative. Acute reactors were defined as patients in whom reagin positivity had reverted to negativity within 6 months of the first positive finding, and chronic reactors as those in whom such positivity had persisted for 6 months or more. It is generally appreciated that acute reactions are likely to arise from virus and other infections and chronic reactions from long-standing serious disorders.

Response from individual physicians was variable as was the intensity of collateral investigations undertaken. During the time of the study 223 forms were returned. In four a differentiation between acute and chronic was not made and these were rejected. Of the remaining 219 forms, 109 referred to acute and 110 to chronic reactors.

The declared criteria were not completely satisfied in a few of the 'acute' cases, which had not been observed for a full 6 months, and some of these would doubtless have been found to become chronic had the patients not defaulted. Also, a number of acute reactions would not have been returned because of default. In addition, as might be expected, the chronic reactors were more intensively investigated than the acute reactors.

Material

SEX

Approximately three-quarters of both groups consisted of female patients, many having been discovered during routine antenatal testing (Table I).

TABLE I *Breakdown according to sex*

BFP Reaction	Acute (109)	Chronic (110)
Total for whom data available	106	110
Male	27	30
Female	79	80
Percentage female	74.5	72.7

RACE

The vast majority of members of both groups were born

in the United Kingdom, which for the purpose of this study has included two patients born in Eire (Table II).

Those born outside the United Kingdom comprised twelve Asians, nine Negroes, three Europeans, and three others.

TABLE II Breakdown according to race

BFP Reaction	Acute (109)	Chronic (110)
Total for whom data available	105	110
Born in UK	91	97
Born outside UK	14	13
Percentage born in UK	86.6	88.2

AGE (Table III)

Most of the acute reactors were below the age of 30 years, and most of the chronic reactors were over 30 years old.

TABLE III Age groups

Age group (yrs)	BFP Reaction	
	Acute (107)	Chronic (108)
0-10	1	—
11-20	18	8
21-30	48	35
31-40	24	29
41-50	5	21
51-60	6	10
61-70	4	3
71-80	—	1
80+	1	1
Total	107	108
Percentage over 30 yrs	37.4	60.2
Percentage under 30 yrs	62.6	39.8

SEROLOGICAL FINDINGS (Table IV)

The findings as regards reagin tests indicate a greater tendency in both groups for the complement-fixation test (e.g. Wassermann) to be positive and the flocculation test (e.g. VDRL) to be negative than the reverse.

In total, the complement-fixation test was positive in 183 cases (95.3 per cent) and the flocculation test in 122 (63.5 per cent).

TABLE IV False-positivity related to complement-fixation and flocculation tests

Tests	Reaction	Reaction				Total (192)	
		Acute (93)		Chronic (99)		No.	Per cent.
		No.	Per cent.	No.	Per cent.		
CFT	Flocculation						
+	+	49	52.7	64	64.7	113	58.8
+	Neg.	37	39.8	33	33.3	70	36.5
Neg.	+	7	7.5	2	2.0	9	4.7
Total known		93	100	99	100	192	100

DURATION OF FALSE POSITIVITY

Of the 109 acute cases, the duration was unrecorded in eight, was less than 10 weeks in 63, and more than 10 weeks in 38.

Of the 110 chronic cases, the duration was unrecorded in eleven, was less than 1 year in forty, and more than 1 year in 59, being from 2 to 11 years in 36.

DISEASE AMONGST REACTORS

Acute reactors

Amongst the 109 acute reactors, disease was noted in 24 patients (Table V).

Of the remainder, nineteen were pregnant, two had had previous attacks of jaundice, and one pregnant woman had a history of rheumatic fever.

TABLE V Disease amongst acute reactors

Type of disease	No. of cases	Nature of disease
Infective	18	Upper respiratory infection 6 Concurrent urinary infection in a diabetic 1 After smallpox vaccination (one in an infant) 4 Herpes simplex 2 Glandular fever 3 Attack of fever with palpable spleen 1 Iritis 1
Autoimmune*	2	Lupus erythematosus 1 Previous history of rheumatoid arthritis 1
Allergic	3	Asthma 1 Penicillin-sensitivity 1 Allergy to nickel 1
Other	1	Heroin addict

*Would presumably have become chronic with the passage of time

Chronic reactors

Disease was noted amongst 33 of the 110 chronic reactors, and in 26 of these 33 an association with the BFP reaction was likely or possible (Table VI, overleaf).

Of the others, nine were known to be pregnant, one other had a history of chorea as a child, and one had had erythema nodosum.

Relationship to blood groups

ABO GROUPS

Comparison with population as a whole

On the 223 forms submitted, data were provided about ABO blood groups for 179 cases (80 acute and 99 chronic). The results are compared with the estimated frequency in English persons in Table VII.

The relative incidence of ABO groups varies markedly in different parts of the world and even in a country as small as the United Kingdom there is some variation between north and south. There is nothing, however, in the above figures to suggest that the ABO

TABLE VI *Disease amongst chronic reactors*

Disease		No. of cases	Nature of disease
Lupus erythematosus	Disseminated, actual or strongly suspected	8	Unspecified 2 Arthropathy and skin rash 1 Hemiplegia and hypertension 1 Haemolytic anaemia 1 ? Myasthenia gravis 1 On basis of demonstrated LE cells 2
	Discoid	4	Pulmonary embolism 1 Haemolytic anaemia 1 Other 2
Other autoimmune or collagen diseases	Actual	6	Autoimmune haemolytic anaemia 1 Thrombocytopenic purpura (also pregnant) 1 Temporal arteritis and abnormal CSF 1 Polyarteritis nodosa (fatal) 1 Rheumatoid arthritis 2
	Suspected	5	Polyarthritis and lymphadenopathy 1 Pyrexia and joint pains treated as endocarditis without improvement 1 Spondylitis and Raynaud's phenomenon 1 Spinal arachnoiditis and pleural effusion—both of unknown aetiology 2
Other possibly associated diseases		3	Bilateral iritis 1 Penicillin urticaria 1 Ill-defined malaise and weakness 1
Other diseases		7	Allergic eczema (one with family history of asthma) 2 Paget's disease 1 Disseminated sclerosis 1 After smallpox vaccination before first test (also pregnant) 1 After polio vaccination 1 Dysfunctional uterine bleeding 1
Total all diseases		33	
Total likely to be or possibly associated with chronic BFP reaction		26	

blood groups in false positive reactors as a whole differ from those in the population at large.

TABLE VII *ABO blood groups in biological false-positive reactors compared with normal persons*

Blood group	Present study		Results in UK population (per cent.)	
	Total	Per cent.	See De Gruchy (1970)	See Worlledge (1972)
A	75	41.9	42.0	41.7
B	15	8.4	8.0	8.6
AB	5	2.8	3.0	3.0
O	84	46.9	47.0	46.7
Total	179	100.0	100.0	100.0

Comparison of acute and chronic reactors

The ABO blood groups in the eighty acute and 99 chronic reactors for whom data are available are compared in Table VIII.

The distribution of ABO blood groups amongst chronic reactors was within the expected range of the UK population. The small differences noted are not statistically significant.

TABLE VIII *ABO blood groups in acute and chronic reactors*

Blood group	Reaction				Per cent. UK population*
	Acute		Chronic		
	No.	Per cent.	No.	Per cent.	
A	31	38.8	44	44.4	41.7-42.0
B	7	8.7	8	8.1	8.0-8.6
AB	2	2.5	3	3.0	3.0
O	40	50.0	44	44.4	46.7-47.0
Total	80	100.0	99	99.9	100.0

*See DeGruchy (1970) and Worlledge (1972)

RHESUS FACTOR

Data on rhesus groups were available in 138 cases. A greater than expected number of rhesus-negative patients above the usual 15 per cent.* was encountered (Table IX, opposite).

*In the series of Schofield (1966), in 2,575 patients attending a venereal disease clinic, the figure was 20 per cent.

TABLE IX Results according to rhesus factor

Rhesus factor	Reaction	
	Acute	Chronic
Total tested	68	70
Rhesus positive	56	52
Rhesus negative	12	18
Per cent. rhesus negative	17.7	25.7

Although the numbers involved are too small for statistical significance, it is noteworthy that, in the thirteen cases of actual or strongly suspected disseminated or discoid lupus erythematosus listed in Tables V and VI, information on the rhesus factor was available for twelve and no less than eight of these were rhesus negative. A high proportion of these results came from one hospital where an intensive study of biological false positive reactions has been made (Catterall, 1972), but it would seem that this matter requires further elucidation.

Other investigations

ERYTHROCYTE SEDIMENTATION RATE

Data were available on 128 patients (Table X).

The erythrocyte sedimentation rate (usually Westergren) was raised in approximately one-fifth of acute reactors but in more than one-half of the chronic reactors, often to a high degree.

TABLE X Erythrocyte sedimentation rate

BFP reaction		Acute	Chronic
No. tested		38	90
Normal		30	44
Abnormal (mm./1 hr)	11-29	4	16
	30-49	4	16
	50-120	—	14
	Range	15-43	11-120
Total abnormal	No.	8	46
	Per cent.	21.1	51.1

SERUM PROTEINS

Gross measurements

Information on the serum proteins was available for 126 patients and abnormal readings were significantly more common in chronic reactors (Table XI).

TABLE XI Serum proteins

BFP reaction		Acute	Chronic
No. tested		34	92
Normal		33	77
Abnormal		1	15
Percentage abnormal		2.9	16.3

The abnormalities were varied and consisted of raised albumin in seven cases (including the one acute reaction), reduced albumin in four, raised globulin in eight, and reduced globulin in four (the normal range of albumin being taken as 3.5-5.2 g./100 ml. and of globulin as 1.7-3.2 g./100 ml.).

Of the fifteen chronic cases with abnormal serum proteins, the erythrocyte sedimentation rate was raised in ten, being above 50 mm./1 hr in five. No overt disease was found in eleven, but systemic or discoid lupus erythematosus was noted in three and auto-antibodies in nine of ten tested—including these three—and there was also one case of temporal arteritis without antibodies. Of those with reduced albumin, liver function tests were otherwise normal in three tested.

Electrophoresis

This was undertaken on sera from 100 patients. Abnormalities by this technique showed no striking difference numerically between acute and chronic cases, although the abnormal findings were more varied in the chronic cases (Table XII).

TABLE XII Electrophoresis

BFP reaction	Acute	Chronic
No. tested	34	66
Normal	27	50
Abnormal	7	16
Percentage abnormal	20.6	24.2

All of the seven acute reactors with abnormalities showed raised gamma globulins. Of the sixteen chronic reactors with abnormalities thirteen showed an increase in gamma globulins, five in alpha-2 globulins (four with gamma globulins in addition), and three in beta globulins (two with gamma globulins in addition); low alpha globulins were reported in one case.

Of the seven acute cases with abnormal findings, the ESR varied between 15 and 43 mm./1 hr in four and was normal in three. Three had no obvious disease, one had had attacks of unexplained fever, one had diabetes and a urinary infection, one penicillin allergy, and one a history of rheumatoid arthritis.

Of the sixteen chronic cases with abnormal findings, the ESR ranged between 26 and 120 mm./1 hr in nine and was normal in seven. No disease was apparent in six but some was evident in ten. Autoimmune tests were positive in nine (including one with no disease) and liver function tests were abnormal in three.

HAEMATOLOGICAL FINDINGS

Haemoglobin content

Taking the lowest normal haemoglobin values to be

13.5 g./100 ml. for males (92 per cent.) and 11.5 g./100 ml. for females (79 per cent.), there were no abnormally low readings amongst 46 acute reactors tested, although one patient was considered to have hypochromia. One patient had a haemoglobin reading of 118 per cent.

Of the 92 chronic reactors tested, reduced readings were found in fourteen (15.2 per cent.), being below 70 per cent. (10.2 g./100 ml.) in two and only 47 per cent. (6.9 g./100 ml.) in one case of autoimmune anaemia in an elderly female. Two patients were noted to have hypochromic anaemia.

Red cell count

27 acute reactors were tested and the red cell count was adjudged to be normal in all (lowest limit of normal regarded as 4,500,000 per cu. mm. for males and 3,900,000 for females).

Of 58 chronic reactors tested, there were three (5.2 per cent.), all female, with abnormal red counts of 3,100,000 to 3,700,000 per cu. mm.; two of these had lupus erythematosus.

White cell count

Of the forty acute reactors tested, one (with no obvious disease) had a white cell count of 12,000 per cu. mm. and one with disturbed liver function a count of 10,500; in 38 the count was normal. In one patient with a count of 8,000, atypical lymphocytes were seen and a diagnosis of glandular fever was confirmed.

Of the 82 chronic reactors tested, one with no overt disease had a white cell count of 14,000 per cu. mm., but in the remaining 81 the total white cell count was normal. Three patients exhibited an eosinophilia of 18, 12, and 6.5 per cent. respectively. One of these patients had polyarthritis with lymphadenopathy and the other two had no obvious disease.

An abnormal platelet count (10,000 per cu. mm.) was reported in one patient with thrombocytopenic purpura, and another (suffering from spondylitis and Raynaud's phenomenon, whose platelet count was not specifically recorded) had been exposed to radiation at Nagasaki in 1945 and had since noticed purpura from time to time.

LIVER FUNCTION TESTS

The findings are summarized in Table XIII.

In all but one of the eighteen cases with abnormalities only one abnormal result was encountered. In half of these it consisted of an increased thymol turbidity and/or flocculation test. In five cases the serum bilirubin was raised, (from 1.2 to 2.4 mg. per cent.). The alkaline phosphatase was slightly raised to 16-18 King-Armstrong units in two and the serum

glutamic oxaloacetic transaminase (SGOT) to 60 units in one patient. Five of the acute and five of the chronic reactors showed no obvious disease.

TABLE XIII *Liver function tests*

<i>BFP reaction</i>	<i>Acute</i>	<i>Chronic</i>
No. tested	29	63
No. abnormal	7	11
Abnormality		
Raised thymol turbidity or flocculation	3	6
Raised serum bilirubin	1	4
Raised alkaline phosphatase	1	1
Raised enzymes	1	—
Urobilinogen in urine	1	1
Percentage abnormal	24.2	17.5

AUTOIMMUNE TESTS

L.E. cells

These were found in one of thirty of the acute group tested. This patient, who underwent a biopsy for a skin rash, had only been observed for 10 weeks and was presumably in reality a chronic reactor. L.E. cells were also found in four of 71 chronic reactors tested; all four had detectable antinuclear factor in the serum.

Serological tests for autoimmune disease

One or more serological tests for autoimmune disease (usually for antinuclear and rheumatoid factor) were performed on sera from thirteen of the acute and sixty of the chronic reactors (Table XIV).

TABLE XIV *Serological tests for autoimmune disease*

<i>BFP reaction</i>	<i>Acute</i>	<i>Chronic</i>
No. tested	13	60
No positive to one or more tests	—	27*
Percentage positive	—	45.0

*Six other patients gave weakly positive results

Amongst chronic reactors, antinuclear factor to a titre of 1/100 or more was found in six and to a lower titre in nine, but 27 (45 per cent.) were found to be reactive to one or more autoimmune serological tests.

Of the 27 with positive tests, seventeen had serious disease (Table VI) and ten had no obvious disease. Only one of the six patients with weakly positive tests had overt serious disease.

The incidence of positivity to autoimmune tests depends to an appreciable extent on the number of different tests performed. In this series the number of different tests made on each specimen is not fully documented. Catterall (1972), in a published study of sixty chronic reactors involving five and in some cases six autoimmune tests (including those for

mitochondrial, smooth muscle, thyroid, and gastric parietal cell antibodies in addition to those for anti-nuclear and rheumatoid factor), found no less than 90 per cent. to be positive to one or more tests.

Summary and conclusions

- (1) A comparison has been made by the British Co-operative Clinical group of 109 acute and 110 chronic false positive reactors to serum tests for syphilis, the great majority of both groups being females born in the United Kingdom.
- (2) Of the acute reactors, 62.6 per cent. were aged 30 years or less, whereas 60.2 per cent. of the chronic reactors were over this age.
- (3) In a considerable proportion of both groups (36.5 per cent. of total), reagin tests of the complement-fixation type were reactive whereas those of the flocculation type were negative; the reverse was the case in only 4.7 per cent.
- (4) Excluding pregnancy, overt disease which could have accounted for the false positivity was noted in 21 of the acute and in 26 of the chronic reactors.
- (5) A study of ABO blood groups showed no significant differences between acute and chronic reactors on the one hand, nor between these together and the population of the United Kingdom as a whole. More than the expected number of chronic reactors, however, were rhesus-negative and a high proportion of the cases of lupus erythematosus included in the study fell into this group.
- (6) Of other investigations undertaken, the erythrocyte sedimentation rate was found to be raised more than twice as often amongst chronic as amongst acute reactors; also higher readings were obtained amongst the former. Similarly, gross measurements of serum proteins were more likely to be disturbed in chronic reactors and, although the frequency of disturbance of the serum proteins as measured by electrophoresis was much the same in the two groups, this appeared to be more varied in the chronic cases. The proportion showing mild disturbance of liver function was similar in both groups.

- (7) Reduced haemoglobin and red cells were not encountered in the acute group but were observed in a small proportion of the chronic group. There was little difference in the white cell count.
- (8) In spite of the relatively small number of different autoimmune tests carried out on the sixty chronic reactors tested, serological abnormalities were nevertheless detected in 45 per cent.

References

- CATTERALL, R. D. (1972) *Brit. J. vener. Dis.*, **48**, 1
- DE GRUCHY, G. C. (1970) 'Clinical Haematology in Medical Practice', 3rd ed., pp. 741-743. Blackwell, Oxford
- KOSTANT, G. H. (1972) *J. Amer. med. Ass.*, **219**, 45
- MOLLISON, P. L. (1967) 'Blood Transfusion in Clinical Medicine', 4th ed., pp. 185-187. Blackwell, Oxford
- MORRISON, A. I. (1966) *Brit. J. vener. Dis.*, **42**, 37
- PASSMORE, R., and ROBSON, J. S., eds (1970) 'A Companion to Medical Studies', vol. 2, pp. 12-13, 31. Blackwell, Oxford
- SCHOFIELD, C. B. S. (1966) *J. med. Genet.*, **33**, 101
- WORLEDGE, S. (1972) In 'Tutorials in Postgraduate Medicine', ed. A. V. Hoffbrand and S. M. Lewis, vol. 2, 'Haematology', pp. 342-343. Heinemann, London

Sujets présentant une réaction biologique faussement positive, aiguë ou chronique, lors des épreuves sérologiques pour la syphilis. Groupes sanguins ABO et autres investigations.

Par le Groupe Britannique de Coopération Clinique

SOMMAIRE ET CONCLUSIONS

- (1) Une comparaison a été faite par le Groupe Britannique de Coopération Clinique chez des sujets présentant une réaction faussement positive (109 fois du type aiguë, 110 fois du type chronique) pour la syphilis, ces sujets étant, pour la grande majorité et pour les deux groupes, nés au Royaume Uni.
- (2) Pour les cas aigus, 62,6 pour cent avaient moins de 30 ans, alors que pour les cas chroniques, 60,2 pour cent avaient dépassé cet âge.
- (3) Pour les deux groupes, une proportion considérable de cas (36,5 pour cent du total) furent réactifs à la classe des tests de fixation du complément alors qu'ils étaient négatifs pour la classe des réactions de flocculation; le contraire s'observa seulement dans 4,7 pour cent des cas.
- (4) En laissant de côté la grossesse, une maladie évidente pouvant être responsable de la fausse positivité fut notée chez 21 des cas aigus et chez 26 des cas chroniques.

(5) L'étude de groupes sanguins ABO ne montra pas de différence significative entre les cas aigus et chroniques ni entre l'ensemble de ces cas et l'ensemble de la population du Royaume Uni. Cependant, un nombre plus grand qu'escompté de positifs chroniques fut Rhésus-négatif et ce fut particulièrement le cas pour une haute proportion des cas de lupus érythémateux appartenant à cette étude.

(6) Parmi les autres investigations entreprises, le taux de sédimentation des hématies fut augmenté deux fois plus souvent qu'escompté et de hauts chiffres furent rencontrés plus souvent pour les cas chroniques que pour les cas aigus. Pareillement, les déterminations grossières du taux des protéines sériques furent plus susceptibles de se montrer modifiées dans les cas chroniques et, quoique la

fréquence de ces modifications des protéines sériques, lorsque on les mesura par électrophorèse, fussent très sensiblement les mêmes dans les deux groupes, celles-ci apparurent plus diversifiées dans les cas chroniques. Dans les deux groupes, la proportion des cas montrant un trouble léger de la fonction hépatique fut identique.

(7) On ne rencontra pas, dans le groupe aigu, de diminutions du taux d'hémoglobine ou du nombre des hématies, mais on en observa un petit nombre dans le groupe chronique. Il n'y eut pas de différence pour la numération des globules blancs.

(8) Malgré le nombre relativement minime des différents tests d'autoimmunité effectués sur les 60 cas chroniques examinés de ce point de vue, des anomalies sérologiques furent néanmoins révélées dans 45 pour cent de ces cas.