

False positive infectious mononucleosis serology in epilepsy

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SYNOPSIS Positive serum tests for infectious mononucleosis (IM) unaccompanied by the clinical syndrome or blood changes characteristic of the disease were detected in 39/177 (22%) mentally subnormal patients investigated with three different commercially available IM slide tests. Slide positivity was still detectable six months later in 27/35 (77%) positive reactors. Positive IM slide tests were significantly associated with anticonvulsant, primarily phenobarbitone therapy and with concomitant elevations of immunoglobulins IgG and IgM. Epstein Barr virus antibodies were not detectable in the sera of 8/38 (21%) positive slide reactors. The implications of these observations are discussed and attention is drawn to the variations in sensitivity, specificity, and comparability of different IM slide tests.

This study of a previously undescribed association between positive infectious mononucleosis (IM) serology and anticonvulsant therapy was prompted by a chance observation in a 31-year-old housewife receiving long-term primidone for idiopathic epilepsy. In April 1972, she was suspected of having IM when she presented to her general practitioner with sore throat and cervical lymphadenopathy. Laboratory investigation revealed a normal blood picture, a positive IM slide test but a negative differential absorption sheep cell test. When identical results were obtained three weeks later it was concluded that the positive IM slide reaction was likely to be of a false positive nature, particularly as the patient was now symptom free. This anomaly led us to examine the sera of a group of randomly selected epileptic outpatients, and similar results were obtained. This report aims to substantiate these preliminary observations and describes exploratory investigations into their possible significance.

Patients studied

One hundred and seventy-seven mentally subnormal patients from the long-stay Ladysbridge Hospital, Banffshire were studied: 115 (63 males, 52 females)

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were epileptics receiving anticonvulsant drugs and 62 (35 males, 27 females) were non-epileptic control patients. The age distribution of the two groups is depicted in the figure. No patient with chromosomal or known immunological abnormality was included. All were in their usual health and in particular none had suffered from sore throat or a glandular fever like illness in the previous six months.

Laboratory Investigations

Haematological examination comprised Coulter 'S' profile (Coulter Electronics Inc., Harpenden, Herts, UK), microscopy of a stained blood film, derivation of a differential white cell count, and a direct anti-globulin test in all subjects.

Serological investigations included initial screening of the sera of all patients with three commercial IM slide kits—Monospot (Ortho Diagnostics Ltd, Raritan, New Jersey, USA); Monosticon (NV Organon, Oss, Holland) and Oxoid (Oxoid Ltd, London, UK). Any serum giving a positive reaction with one or more of these tests was then subjected to a conventional differential absorption test (Davidson, 1967).

Serum immunoglobulins IgA, IgG, and IgM were measured on single diffusion quantitative radial immunoassay Partigen plates standardized with reference sera (Behringwerke AG, Marburg/Lahn, West Germany).

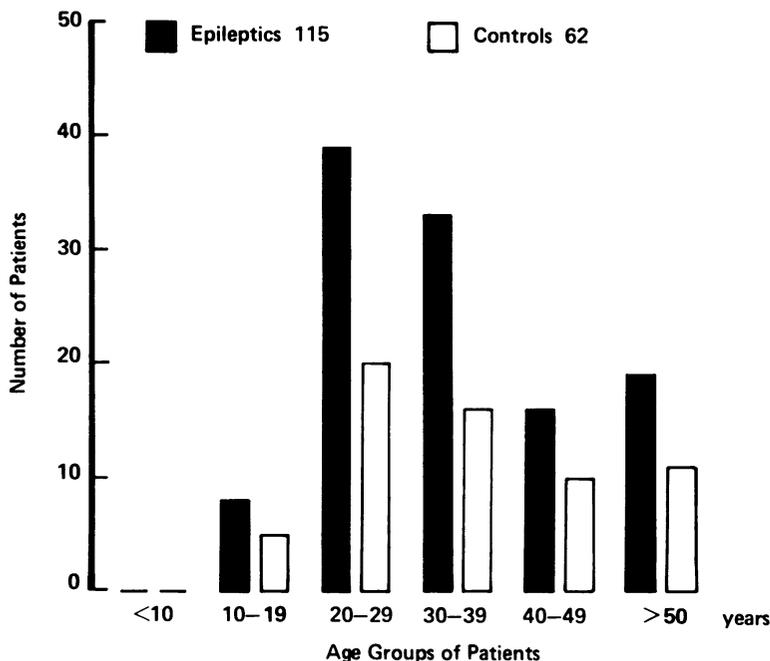


Figure Age distribution of the patients studied.

Epstein-Barr virus (EBV) antibody titres were determined using a fluorescent antibody technique (Henle and Henle, 1966).

Results

INCIDENCE AND PERSISTENCE OF IM SLIDE TEST POSITIVITY

Thirty-two epileptics (19 males, 13 females) and seven control patients (1 female, 6 males) had positively reacting sera with one or more of the slide tests. While slide reactivity was unrelated to age or sex the incidence of slide test positivity in epileptics (28%) compared to controls (11%) was significantly increased ($\chi^2 = 5.39$, $P < 0.02$). Such a high rate of positivity in controls was quite unexpected.

Six months after initial screening, 35 of the 39 patients with positive slide tests were re-investigated. In the epileptic group 24/28 patients remained positive in contrast to 3/7 in the control group ($P = 0.06$, by two-tailed exact test; Armitage, 1971).

These findings confirm our initial observations and suggest a possible link between the occurrence and persistence of IM slide positivity and anti-convulsant therapy.

COMPARISON OF THE DIFFERENT SLIDE TEST SENSITIVITIES

Of considerable interest and practical importance is the difference in the number of positive reactions

given by the three different slide tests used to detect IM heterophile antibody. Monospot was the most sensitive reagent, identifying 27/35 (70%) sera initially positive and 25/27 (89%) positive on re-examination six months later. The Oxoid reagent detected 19/29 (49%) and 13/27 (49%) while Monosticon detected 18/39 (46%) and 11/27 (41%). The higher rates of detection of Monospot over the other two reagents were highly significant ($\chi^2 = 11.8$ and 15.5 ; $P < 0.001$). The concurrence of the results, measured by correlation coefficient analysis, comparing Monospot with Oxoid and Monospot with Monosticon was high ($r = 0.77$; $r = 0.74$), but the correlation between the two weaker detectors was relatively low ($r = 0.33$). Such discrepant findings emphasize the need for further study and evaluation of factors influencing the sensitivity and specificity of these reagents in diagnostic serology.

HAEMATOLOGICAL EXAMINATION AND SLIDE TEST POSITIVITY

Only two abnormal white cell patterns emerged, a mild neutropenia in those receiving anticonvulsant drugs ($\chi^2 = 4.20$, $P < 0.05$) and a high incidence of eosinophilia (> 440 eosinophils $\times 10^9/l$) in both control (13%) and epileptic (14%) patients. Atypical mononuclear cells were not detected in significant numbers in the peripheral blood of any patient and we were unable to confirm the previously reported association of phenytoin hypersensitivity and the

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presence of atypical mononuclear cells (Wood and Frenkel, 1967). The direct antiglobulin test was consistently negative in all subjects.

DIFFERENTIAL ABSORPTION AND SLIDE TEST POSITIVITY

All sera giving a positive reaction with one or more of the IM slide tests were found to have low saline heterophile antibody titres (geometric mean 1:34). None had an absorption pattern diagnostic of IM. On retesting six months later, similar results were obtained in all but one patient whose titres, previously 1:16, had developed a pattern characteristic of IM (saline titre 1:256; post absorption titres with guinea pig kidney and with ox cells 1:128 and 1:32 respectively). This patient, a 37-year-old woman receiving 90 mg of phenobarbitone daily, remained asymptomatic throughout with no clinical or haematological evidence of IM.

ANTICONVULSANT AND SLIDE TEST POSITIVITY

Multiple drug regimens were commonly used in the treatment of these epileptic patients and hence the rôle and contribution of any one anticonvulsant was difficult to assess. Only 5/32 positive epileptic slide reactors were not receiving phenobarbitone or primidone, the therapeutic effect of which depends upon its metabolic degradation to phenobarbitone (Bogan and Smith, 1968), although a significant association could be demonstrated only between IM slide positivity and phenobarbitone usage (table I).

No. of Patients	Anticonvulsant Therapy			
	Pheno-barbitone	Primi-done	Pheny-toin	Sulthiame
Slide positive (39)	26	13	10	4
Slide negative (138)	53	27	14	13
χ^2 association between slide positivity and different drugs	4.56 P < 0.05	2.25	0.85	0.01

Table I *Drug usage and IM slide positivity*

IMMUNOGLOBULINS AND SLIDE TEST POSITIVITY

Only minor differences in mean immunoglobulin levels (calculated from logarithmically transformed data (Hobbs, 1970)) were demonstrated in positive and negative slide reactors in both epileptic and control groups (table II). Although similar minor changes have been reported in convalescent IM patients (Sutton *et al*, 1973) no definite conclusions can be drawn from these figures because comparable disturbances have also been described in both epileptic and mentally defective patients (Sorrell

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No. of Patients	Immunoglobulin Levels (mean \pm standard deviation) \log_{10} mg/dl		
	IgG	IgA	IgM
Epileptic			
Slide positive (32)	3.154 \pm 0.164	2.301 \pm 0.296	2.259 \pm 0.247
Slide negative (83)	3.117 \pm 0.118	2.248 \pm 0.316	2.300 \pm 0.302
Control			
Slide positive (7)	3.101 \pm 0.074	2.508 \pm 0.113	2.296 \pm 0.184
Slide negative (55)	3.040 \pm 0.118	2.321 \pm 0.178	2.208 \pm 0.198

Table II *Immunoglobulin levels*

et al, 1971; Grob and Herold, 1972). If, however, sera with elevated levels of IgG and/or IgM (IgG > 16 g/l; IgM > 2 g/l) are considered (table III) a definite association was found between IM slide positivity and concomitant elevations in both. Such findings are consistent with secondary humoral response to infection being associated with a positive IM slide test.

No. of Patients	IgG alone > 16 g/l	IgG and IgM both raised	IgM alone > 2 g/l
Slide positive (39)	6	10	13
Slide negative (138)	14	12	32

Table III *The patterns of IgG and IgM response associated with IM slide positivity*

EBV ANTIBODY TITRES AND SLIDE TEST POSITIVITY

On initial testing EBV antibody titres were generally low (table IV). Surprisingly, the mean titre in positive slide reactors was lower than that in the negative group, and no difference was found in the titres of paired sera from positive reactors over a period of six months. IM slide test positivity in these patients did not therefore seem to indicate recent or continuing EBV infection. Such a conclusion is substantiated by finding no detectable EBV antibody in eight positive reactors (mean age 30, range 10 to 58 years).

No. of Patients	EBV Antibody Titres			
	Negative	1:10-1:320	1:320	Geometric Mean
Slide positive (38)	8	28	2	19
Slide negative (138)	12	117	9	30

Table IV *EBV antibody titres*

Discussion

In this controlled study we have confirmed our initial

observation of an association between anticonvulsant therapy and IM slide test positivity. This unexpected serological finding has been shown to be of a 'false positive' nature, being found in the absence of clinical or haematological manifestations of the disease and unaccompanied by a positive conventional differential absorption reaction. Furthermore, the pattern of EBV antibody response in positive reactors was not indicative of primary or reactivated infection with the virus, the presumed aetiological agent in IM (Epstein and Achong, 1973) and an anamnestic resurgence of true IM heterophile antibody was likewise excluded. Thus, while a relationship between false positive IM slide reactions and anticonvulsant therapy is established, the phenomenon and causal mechanisms remain unexplained.

A simple and direct drug reaction would appear unlikely as preliminary study of a small group of patients with acute barbiturate intoxication yielded negative findings and, despite the prevalence of barbiturate usage in the general population, similar observations have not previously been noted. On the other hand, an indirect drug effect might be considered. Anticonvulsant drugs are mildly immunosuppressive, capable of inducing alterations in both cellular and humoral responses (Sorrell *et al*, 1971; Grob and Herold, 1972) with the consequent defective immunosurveillance leading in rare instances to malignant transformation within the reticulo-endothelial system (Editorial, 1971). In the epileptic patients we have studied, anticonvulsant therapy may thus be altering immunoregulatory mechanisms, thereby favouring atypical or even inappropriate humoral responses which included the development and persistence of an IM slide detectable heterophile antibody. Although the nature of the underlying immunogenic stimulus has not been identified, our findings indicate that it is not the potentially oncogenic EB virus (Epstein and Achong, 1973).

The high incidence (11%) of slide positivity in controls was unexpected and remains unexplained. Such an incidence is much higher than that (1 to 2%) usually associated with this diagnostic technique (Glade, 1972). The specificity of slide testing, however, has generally been assessed by comparing the results in known cases of IM with those in randomly selected controls. False positive tests have been reported in patients with viral infections including infectious hepatitis, cytomegalovirus, adenovirus, and rubella (Seitanidis, 1969; Wahren, 1969; Phillips, 1972) and in one of our own unpublished studies relating to some 1658 specimens sent to this laboratory from patients suspected on clinical grounds of having glandular fever, 65 (3.9%) gave an unexplained but consistently positive slide reaction with a negative differential absorption test.

Institutionalized populations, in contrast to open communities, have increased exposure to infection which gives rise to appreciable alterations in immunoglobulins (Thom and McKay, 1972). The high background incidence of IM slide positivity present in our control group of patients may thus be a reflection of an antibody response to some unidentified antigenic stimulus present within the community, most probably of an infective nature. However, serological testing for complement fixing activity to influenzae A, B, and C, Sendai, adenovirus, respiratory syncytial virus, measles, herpes simplex, and *Mycoplasma pneumoniae* antigens did not provide any diagnostic pointers.

Apart from their interesting hypothetical implications our findings are not without practical relevance in the field of diagnostic serology. Our failure to support and confirm persistent IM slide positivity by conventional differential absorption and EBV antibody tests indicates a need to reassess the principles on which the sensitivity and specificity of the IM slide techniques are based. Furthermore, the results obtained with the three commercial reagents were so discrepant as to question their comparability and draw attention to a need for their standardization.

Finally, we emphasize that in the clinical setting of an epileptic patient presenting with sore throat, fever, and lymphadenopathy, a triad that includes the unusual complication of pseudolymphoma, a positive IM slide reaction requires cautious interpretation if it is not supported by additional evidence of IM or EB virus infection.

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