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CASE REPORTS

Toxoplasmosis and systemic lupus erythematosus

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Abstract

The toxoplasma serological status of 50 patients with systemic lupus erythematosus (SLE) was compared with that of 50 healthy controls; high titres of toxoplasma antibody were significantly more common in the patients with SLE. These titres did not correlate with any of the routinely measured indices in SLE nor with the patients' prior treatment. A case history is used to illustrate the difficulty in diagnosing toxoplasmosis in the presence of SLE.

Toxoplasmosis and systemic lupus erythematosus (SLE) produce similar symptoms, such as lymphadenopathy, arthralgia, and fever, and increased awareness of these conditions and refinements in 'diagnostic markers' have led to both conditions being recognised more often. We present a case history to highlight the problems faced when attempting to diagnose toxoplasmosis in the presence of SLE. The persistently high toxoplasma antibody levels found in the patient described prompted this study of the toxoplasma serological status in a cohort of patients with SLE.

Case report

A 28 year old Ghanaian woman presented in 1976 with a facial rash, periorbital oedema, mucosal ulceration, and widespread papular lesions associated with tachycardia and fever (39°C). Stevens-Johnson syndrome was considered, and she reponded well to steroids, but no immunological investigations were performed. Within six months of presentation she developed alopecia and polyarthritis, involving wrists, metacarpophalangeal joints, and proximal interphalangeal joints, and was treated with non-steroidal anti-inflammatory agents.

In 1979 lethargy and arthralgia were a problem and examination showed widespread tender lymphadenopathy and synovitis of the wrists and left knee. Investigations disclosed abnormal lymphocytes on a peripheral blood film, a negative Paul-Bunnell test, reactive changes in bone marrow and lymph node biopsy specimens, a negative sickle test, erythrocyte sedimentation rate 60 mm/h, and normal hand radiographs. Toxoplasma serology showed a Sabin-Feldman dye test titre 1/16000 and IgM immunofluorescence antibody titre 1/80 (confirmed by repeat testing). Acute toxoplasmosis was diagnosed and treated with pyrimethamine and sulphadiazine for two months followed by spiramycin for two months with limited clinical improvement. Further investigations showed: antinuclear antibody titre 1/6000 (IgG class), 34% DNA binding (normal <5%), rheumatoid factor tests negative, polyclonal increases in serum IgA, IgM, and IgG, and a skin biopsy specimen which showed a positive lupus band test. Over the next three years she was treated with hydroxychloroquine and subsequently steroids, leading to resolution of the lymphadenopathy and improvement in joint symptomatology. At this time the toxoplasma serology showed a dye test titre 1/1024 and IgM antibody titre <1/10.

In 1986 she developed painful vasculitic leg ulcers and the investigations showed antinuclear antibody titre 1/6400, double stranded DNA (dsDNA) antibodies negative, and C3dg 18 U/ml (moderate increase). She was successfully treated with steroids, and at present she has occasional joint symptoms, Raynaud's phenomenon, no lymphadenopathy or signs of ocular toxoplasmosis, but continues to have vasculitic lesions on her legs. Currently, toxoplasma serology shows a positive dye test of 1/4096 and toxoplasma IgM is negative.

The association of antibodies to *T gondii* with SLE was further investigated and the prevalence of positive toxoplasma serology in a group of patients with SLE determined.

Patients and methods

Fifty patients with SLE fulfilling four or more American Rheumatism Association revised criteria were randomly selected and investigated for serological evidence of toxoplasma infection.[1] Serum samples were obtained from 50 controls matched for age and sex from two sources—namely, serum samples submitted to the Nottingham Public Health Laboratory (NPHL) for antenatal rubella immune status screening or serum samples from patients/relatives attending the accident and emergency department with minor injuries.

Serum samples were assayed at the NPHL by a commercially available latex agglutination test—Toxoreagent, 'Eiken'.[2] Further toxoplasma serology, consisting of a haemagglutination test, Sabin-Feldman dye test, and IgM enzyme linked immunosorbent assay (ELISA), was performed at the toxoplasmosis unit of the Regional Public Health Laboratory, Leeds.[3 4] IgG class rubella antibody was measured by haemagglutination-inhibition on patient and control sera at the NPHL.

Immunological studies comprised: IgM and IgG class antinuclear antibodies by indirect immunofluorescence using a rat liver substrate; IgM and IgG class antibodies to dsDNA by

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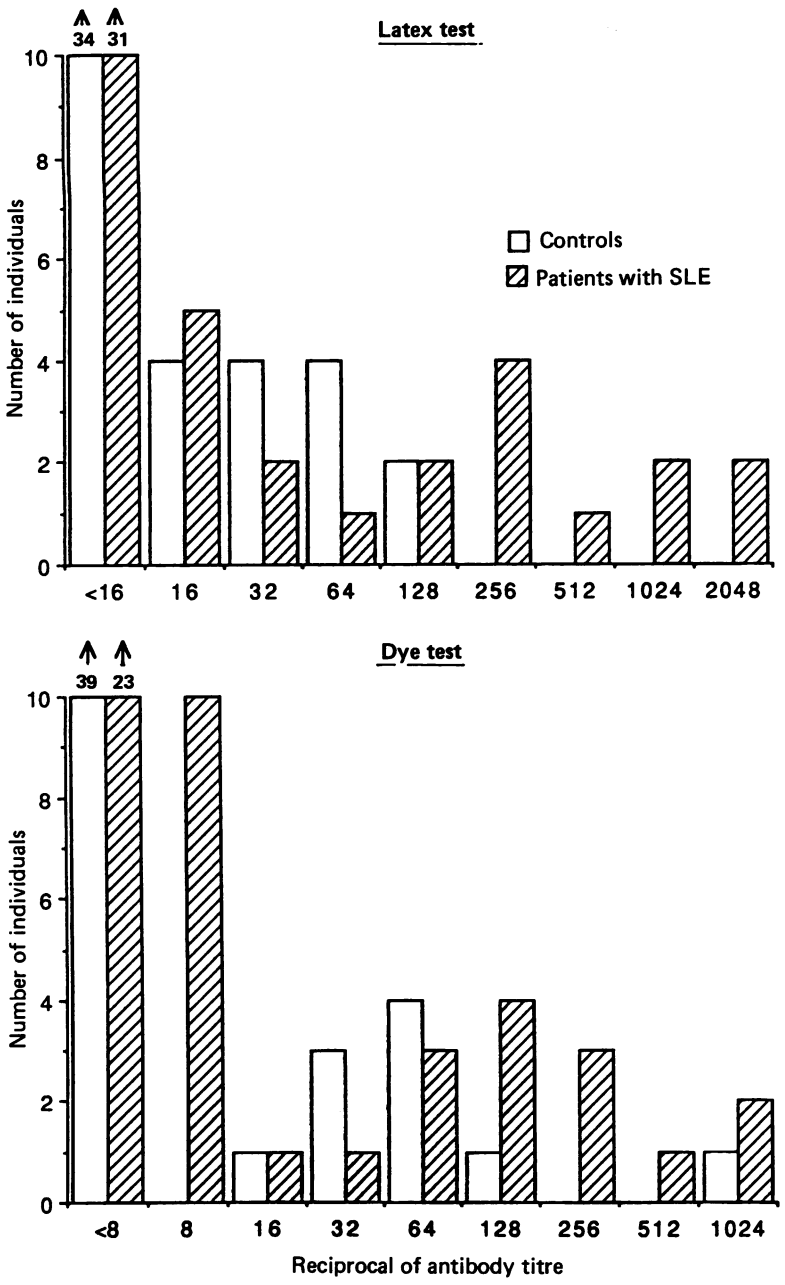

Histograms giving distribution of toxoplasma serology (latex test and dye test) in patients with systemic lupus erythematosus and in controls.

indirect immunofluorescence with *Crithidia luciliae* as substrate; antibody to soluble cellular antigens by countercurrent immunoelectrophoresis; IgA, IgM, and IgG class rheumatoid factor, using highly purified human Fc IgG as a substrate, by ELISA[5]; IgM and IgG class antiphospholipid antibody by ELISA[6]; the complement degradation product C3dg by double decker rocket immunoelectrophoresis; serum IgG, IgM, and IgA by turbidimetric method on centrifugal fast analyser.

Clinical details and treatment regimens were obtained from patients' hospital notes. Statistical analysis was performed using χ^2 and Z values (Mann-Whitney U test).

Results

Close agreement between toxoplasma serological tests—namely, the dye test, latex agglutination test, and haemagglutination test, was noted for all individuals studied. The figure shows the concordance between the latex and dye tests and table 1 illustrates the agreement between the toxoplasma serological tests in those patients with SLE with a latex agglutination test titre $\geq$1/128. Significant toxoplasma antibody titres were taken as $\geq$8 IU/ml in the dye test, $\geq$1/16 in the latex agglutination test, and $\geq$1/64 in the haemagglutination test.[3] In the dye and latex tests 20% and 29% of control sera were positive for toxoplasma antibody respectively. The cor-

Table 1: Toxoplasma serology in patients with systemic lupus erythematosus with high titre antibody ($\geq$1/128)

Patient No	*Dye test titre*	*Haemagglutination test*	*Latex test titre*	*IgM ELISA* units*
7	128	256	128	0
8	128	$\geq$1024	1024	12
18	128	1024	256	0
20	1024	$\geq$1024	512	71
21	64	$\geq$1024	1024	1
22	512	128	128	0
24	1024	$\geq$1024	2048	10
30	128	512	256	6
31	256	$\geq$1024	2048	3
32	64	1024	256	5
46	256	$\geq$1024	256	0

*ELISA=enzyme linked immunosorbent assay.

Table 2: Results of immunological tests on the serum samples of patiens with systemic lupus erythematosus with high levels of toxoplasma antibody (titre $\geq$1/128)

Patient No	*Age (years)*	*IgG* ANAs† (titre)*	*Immuno-globulins* (g/l)*	*APL*† antibodies (units)*		*Antibodies to dsDNA (titre)*	*ENAs†*	*C3d* (units/ml)*	*HAI† rubella (titre)*	*Rheumatoid factors* (units)*		
				IgM	*IgG*					*IgG*	*IgM*	*IgA*
7	42	800	N†	N	N	N	RNP+	10	2000	1·9	4·4	22·5
8	66	200	N	N	N	N	N	12	30	2·8	33·1	12·0
18	76	10	N	N	N	N	N	7	120	0·7	0·3	6·0
20	42	400	G 19·04	N	N	N	RNP+	12	120	1·0	0·9	52·5
21	71	800	N	N	N	N	SSA+/B+	13	250	2·0	5·9	200·0
22	39	10	N	N	N	N	N	14	120	5·7	1·8	14·5
24	83	10	N	N	N	N	N	7	120	0·6	0·6	29·0
30	68	100	N	N	N	N	N	10	30	0·9	3·6	5·5
31	49	1600	G 41·9	N	N	G 640	Thymus+	24	—	6·0	15·3	92·0
32	61	3200	N	9	N	N	N	—†	15	—	—	—
46	17	800	N	N	N	G 1280 M 640	N	18	120	0·6	1·0	28·0

*Normal ranges—IgA ANAs <10=not significant; immunoglobulins: IgA 1·25–4·25, IgG 5·00–16·00, IgM 0·50–1·75=normal ranges; APL antibodies IgM 3, IgG 6=negative; C3d $\leq$12=negative; rheumatoid factors: IgG <2·5, IgM <3·8, IgA <17 arbitrary units=negative.
†ANAs=antinuclear antibodies; APL=antiphospholipid; ENAs=extractable nuclear antigens; HAI=haemagglutination inhibition; N=normal/absent; —=not tested.

Table 3: Distribution of rubella antibodies in patients with systemic lupus erythematosus (SLE) and in controls

	Rubella antibody reciprocal titres								
	<15	15	30	60	120	250	500	1000	2000
Patients with SLE	6	3	6	5	14	12	0	2	1
Controls	2	3	11	13	13	6	2	0	0

Table 4: Previous treatment regimens in patients with systemic lupus erythematosus related to levels of toxoplasma antibody

Toxoplasma LAT* reciprocal titre	Nil or HC*	Pred* +HC	Aza*	Cyclo*	Not known
<16	14	4	6	1	6
16–64	3	2	1	1	1
>128	4	3	3	0	1

*LAT=latex agglutination test; HC=hydroxychloroquine; pred=prednisolone; aza=azathioprine; cyclo=cyclophosphamide.

responding figures for the patients with SLE were 51% and 38%. The difference in positive toxoplasma serology results for the patients with SLE and controls was highly significant for the dye test values ($p<0{\cdot}001$) but not significant for the latex agglutination test ($p>0{\cdot}06$). The proportion of strong positives (titre $\geq 1/128$) was significantly greater in patients than in controls for both the dye ($p<0{\cdot}01$) and latex ($p<0{\cdot}005$) tests. One of the patients with SLE, a strong positive, had a significant level of IgM class toxoplasma antibody, indicating recent infection, whereas none of the control group had evidence of recent infection.

Table 2 gives the immunological test results for the patients with SLE with high levels of toxoplasma antibody (titre $\geq 1/128$). There was no relation between these SLE indices and the high positive toxoplasma antibody levels. Only two of 11 patients with high toxoplasma antibody levels had increased serum immunoglobulins compared with 25 out of the other 39 patients ($p>0{\cdot}01$). Table 3 compares the distribution of rubella antibodies in patients with SLE with those in the controls. No significant difference was seen between the two groups ($p>0{\cdot}1$). The serum immunoglobulin concentrations in the control group showed no abnormal IgA or IgG values, but eight controls had polyclonal increase in IgM.

Table 4 summarises the treatment regimens for the patients with SLE. There was no significant difference in treatment between those patients with negative, low, or high levels of toxoplasma antibody.

Discussion

The incidence of toxoplasmosis is increasing, especially in immunosuppressed patients secondary to treatment or HIV infection.[7] Increased awareness of toxoplasmosis will lead to more requests for serological testing upon which the diagnosis rests. Most patients with toxoplasmosis are asymptomatic, whereas others experience a glandular fever like illness, and about 1% of the British population/year show evidence of seroconversion.[8] Such symptoms may be difficult to differentiate from a flare of disease activity in lupus patients, as exemplified by the case history. That patient received lengthy courses of two different therapeutic regimens but remained symptomatic, and only then was the diagnosis of SLE considered. This was based on high titre antinuclear antibodies, antibodies to dsDNA, and a characteristic skin biopsy immunofluorescence pattern. Resolution of symptoms occurred on treatment with hydroxychloroquine and steroids. The patient's presenting symptoms might well have been attributable to SLE, and the absence of immunological investigations at presentation is regrettable. It is notable that high toxoplasma antibody titres continue nine years after the putative diagnosis of toxoplasmosis but the IgM toxoplasma antibody levels have fallen.

This study indicates a significant difference in the number of positive titres of toxoplasma antibody in patients with SLE measured by the dye test compared with healthy controls. It would be unwise to place too much emphasis on this point, however, because the difference between the two groups is mostly due to a greater number of patients having low toxoplasma antibody titres (1/8). More importantly, the number of strong positives by the latex agglutination and dye tests in the patients with SLE is significantly higher than in the controls (11 out of 50 compared with two out of 48 in the latex agglutination test). Latex agglutination test titres of $\geq 1/128$ were chosen as a cut off because in Nottingham this is the screening level at which serum are samples normally sent to the toxoplasma reference laboratory for further investigation. Such a method is designed to identify patients with relatively recent disease which may be clinically significant. The dye test is still the method of choice as false positives have not been described, but it has the limitation of using live toxoplasma organisms. The latex agglutination test is a new method, which is easily carried out in non-specialised laboratories. We investigated a spectrum of possible serological variables in patients with SLE which might interfere with the measurement of toxoplasma antibody levels. In particular, the absence of correlations between toxoplasma antibody positivity and the presence of autoantibodies such as rheumatoid factors and antinuclear antibodies is notable because rheumatoid factors are well known for their ability to interfere with serological assays,[9] and antinuclear antibodies may give false positive toxoplasma antibody results when the latter is measured by an immunofluorescent technique.[10] Plasma C3dg may be considered to be an indirect measure of immune complexes, but the former showed no correlation with the dye or latex tests. It seems that the raised toxoplasma antibody levels are not part of a polyclonal antibody response in the lupus patients. Rubella titres were not significantly higher in patients than in controls, and particularly not in those with high levels of toxoplasma antibody. Therefore, the raised toxoplasma antibody titres are unlikely to be part of an anamnestic reaction,

but this remains unproved. It has been shown that adults may have naturally occurring antibodies to toxoplasma antigens and it may be worth investigating the role of these, if any, in lupus patients.[11] It is notable that there are several cases reported of toxoplasmosis in association with polymyositis, and small improvements in one such case occurred after treatment for toxoplasma infection.[12] Infection is a well described cause of both morbidity and mortality in patients with SLE.[13] Although studies have not looked at toxoplasma infection, possibly some symptoms in lupus patients might indeed be related to such infection. It may therefore be of value to measure toxoplasma antibody levels in such instances. Retrospective analysis of patients' notes did not show any obvious clinical manifestations of toxoplasmosis in the patients with high antibody titres. The situation is further complicated because IgG toxoplasma antibody titres, as measured by the dye or latex agglutination tests, may remain raised for years, whereas ophthalmic toxoplasmosis may be associated with only moderate antibody titres. Recent infection is most easily detected by measuring levels of IgM toxoplasma antibody.

Studies have shown that steroid use is a major cause of the increased infection rates in lupus patients. Our patients with either negative, low, or high toxoplasma antibody levels had not been treated differently. To assess fully the significance of immunosuppressive treatment in SLE and toxoplasmosis an untreated SLE control group would be required.

In conclusion, our results suggest that patients with SLE may be more likely to have positive toxoplasma serology, and that some have particularly high antibody titres. There is no evidence that the results are false positives and the increased toxoplasma antibody levels do not seem to be part of a polyclonal increase in immunoglobulins. Our findings of higher than expected toxoplasma antibody levels in lupus patients may be important in the interpretation of toxoplasma serology in such subjects. The possibility that some symptoms and signs in patients with SLE may be due to toxoplasma infection should not be ignored.

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