Clin. exp. Immunol. (1983) **54**, 723–730.

Anti-globulins and circulating complexes in early rheumatoid arthritis

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(Accepted for publication 12 August 1983)

SUMMARY

The importance of immunological parameters such as anti-globulins, anti-RANA and circulating immune complexes in rheumatoid arthritis (RA) has been studied by examining patients with early disease who are attending general practitioner clinics with joint pains for the first time. Anti-RANA, and IgG and IgM anti-globulins were detectable in the serum at the earliest time we were able to examine the patients. The anti-globulins had specificity for both rabbit and human IgG from the outset. Immune complexes were similarly raised in early disease. From these patients with early joint pains we were able to predict, by means of multivariant discriminant analysis of the laboratory data obtained from the first serum sample, between those who would develop into patients with classical or definite RA at 1 year and those who would have non-inflammatory joint disease.

Keywords anti-globulins discriminant analysis rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a disease with many immunological ramifications. Perhaps the most pervasive of these is the heightened response to self-IgG and raised levels of such anti-globulins, or rheumatoid factors (RFs), can be demonstrated in virtually all patients if appropriate methods of assay are employed (Torrigiani & Roitt, 1967; Hay, Nineham & Roitt, 1975). This has led many authors to the view that IgG autosensitization provides the major, if not the only, driving force underlying the pathogenesis of this disorder (Winchester, Agnello & Kunkel, 1970; Roitt *et al.*, 1982). Another common feature of RA is the presence of circulating immune complexes (CICs) which are generally thought to be composed largely of anti-globulins (Hay, Nineham & Roitt, 1979). It is considered by some that complexes are generated essentially in the joint tissue with the smaller ones escaping into the circulation (Hay *et al.*, 1979) while others maintain that they originate in extra-articular sites and deposit from the blood into the joints in a form of 'serum sickness' (Bacon, 1979).

Recently, interest in the possibility of virus involvement in the disease has been re-awakened by the finding of high titre antibodies directed against an Epstein–Barr (EB) virus-induced nuclear antigen (RANA) in RA patients as compared with controls and subsequently it has been reported that T cell control of EB virus infection of B lymphocytes is defective in RA (Depper & Zvaifler, 1981). Whether this is symptomatic of a general defect in the handling of viruses because of some T cell abnormality, or whether EB virus itself might be a trigger for the onset of disease, has not been resolved.

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It would clearly be of interest to determine the relative times of appearance of these phenomena in individual patients to see if any one parameter could be identified as playing a primary role. Accordingly, through co-operation with general practitioners, we attempted to find patients with the earliest possible manifestation of the disease and in the present communication we report on their anti-globulins and CICs. We have already described the status of these patients with respect to anti-RANA (Male *et al.*, 1982).

MATERIALS AND METHODS

Patients. Twenty-five general practices in and around London agreed to refer all patients presenting with joint pains for the first time. The average period from the onset of first symptoms was 11 weeks with a range of 2 days to 6 months. All patients were examined by one rheumatologist and followed at regular intervals by the same physician for at least 1 year.

Patients were initially classified independently of serological tests and were then re-classified with the clinical radiological and serological data at the end of one year into the following groups. (1) RA, definite and classical rheumatoid arthritis according to the ARA criteria. (2) Transient synovitis (TS). Synovitis lasting less than 6 weeks. (3) Non-rheumatoid inflammatory joint disease (IJD) e.g. psoriasis, SLE, etc. (4) Non-inflammatory joint disease (NIJD) e.g. osteoarthritis.

One patient was removed from the definite and classical group because she was persistently seronegative, and probably associated with primary billiary cirrhosis. Another was re-classified as RA on the basis of radiological findings.

Anti-globulins. Conventional RFs were measured by the agglutination of sheep cells coated with rabbit IgG (RAHA;Fuzizoki) and of latex particles bearing human IgG (Hyland). IgG anti-globulins were assayed by solid phase techniques. Anti-globulins with specificity for rabbit IgG were allowed to bind to plastic tubes coated with rabbit IgG and detected with the $F(ab')_2$ fragment of radiolabelled rabbit anti-human IgG Fd (Jones, 1982). For the assay of human specific anti-globulins, human Fc was bound to the tubes and a labelled anti-human IgG Fd used as the second antibody (Jones, 1982).

CIC. For the polyethylene glycol (PEG) method, complexes were precipitated from serum by 2% PEG and the IgG in the precipitate measured by laser nephelometry (Hudson & Hay, 1981). The solid phase C1q binding test involves the adherence of complexes to C1q coated tubes and their estimation with labelled anti-human IgG (Hudson & Hay, 1981).

Statistics. Tests for significant differences between means were made using Student's *t*-test for continuous data and the Mann–Whitney test for discontinuous data. Correlations were similarly established using either Pearson's correlation co-efficient or Spearman's rank correlation co-efficient ρ. Multivariant analysis using discriminant functions was performed using the Statistical Package for the Social Sciences, Version M, Release 9·0 on the Amdahl computer at the University of London Computer Centre. The analysis was assessed in terms of specificity and sensitivity, where specificity is defined as (1 − the probability of a falsely positive diagnosis), and sensitivity is (1 − the probability of a falsely negative diagnosis) (Armitage, 1970).

RESULTS

Anti-globulins

It should be emphasized that the patients, who were examined shortly after their first presentation to the general practitioner with joint pains, were classified initially into different groups entirely on the basis of clinical criteria, without knowledge of any laboratory investigations. It was striking (one might say reassuring!) that with one exception, the classical agglutinating RFs were only detected in early RA and those in whom the disease was found to be already established. Fifteen of twenty-five (60%) of early RA patients and four of six established RA gave latex titres of 20 or higher whereas all other groups had titres of less than 20 (Fig. 1a). Titres of 40 or more in the RAHA test were found in 14 of 26 (54%) of early rheumatoids and six of nine established RA but in none of

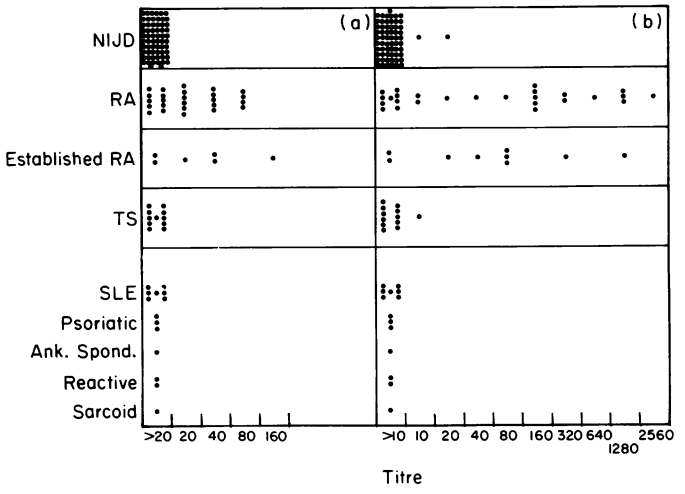

Fig. 1. Titres of classical RFs using (a) latex coated with human IgG and (b) sheep cells bearing rabbit IgG (RAHA) in patients with 'early' joint pains.

the other groups (Fig. 1b). Altogether 16 of 26 (62%) of the early and seven of nine of established RA sera gave titres of 20 or higher in the latex and/or 40 or more in the RAHA tests, the correlation between the two tests being $\rho = 0{\cdot}80$ ($P = 0{\cdot}001$). Patients with established RA also had RF but virtually all the other groups examined were completely negative.

These sera were also examined for IgG anti-globulins, again against human and rabbit IgG. Very similar rates of seropositivity were found to the agglutination assays, with 73% of early RA patients having increased anti-rabbit anti-globulins (Fig. 2b) and 69% with anti-human anti-globulins (Fig. 2a) compared with age and sex matched normal controls. There was a close relationship between the IgG anti-globulins of each specificity ($r = 0{\cdot}94$, $P = 0{\cdot}0001$), but less correlation was found between the agglutination results (RAHA) and the IgG anti-globulins (human IgG $\rho = 0{\cdot}63$, $P = 0{\cdot}001$; rabbit IgG $\rho = 0{\cdot}50$, $P = 0{\cdot}006$). When the results of the two IgG anti-globulin assays were

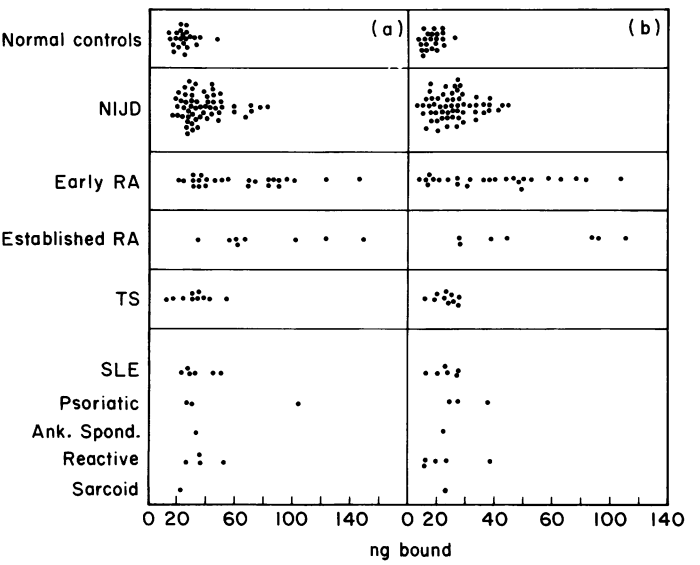

Fig. 2. IgG anti-globulins directed against (a) human and (b) rabbit IgG determined by solid phase assay in patients with 'early' joint pains.

combined, nearly all (88%) of the early RA patients were found to have raised levels of anti-globulin. Lower but still significant correlations were found between the latex agglutination titres and the IgG anti-globulins (human specificity, $\rho = 0{\cdot}43$, $P = 0{\cdot}02$; rabbit specificity $\rho = 0{\cdot}39$, $P = 0{\cdot}03$). Some of the patients with NIJD were also found to have raised amounts of anti-globulin but the early RA patients had significantly higher levels of both anti-rabbit IgG ($P = 0{\cdot}002$) and anti-human IgG ($P = 0{\cdot}001$). Patients with TS or NIJD rarely had raised anti-globulins. The titre of anti-RANA antibody showed some association with the IgG antiglobulins (human specificity, $\rho = 0{\cdot}50$, $P = 0{\cdot}007$; rabbit specificity, $\rho = 0{\cdot}53$, $P = 0{\cdot}004$).

CIC

CIC were estimated by two methods, nephelometric quantitation of IgG complexes precipitated with 2% PEG and by radioimmunoassay of IgG complexes bound to solid phase C1q. Immune complexes were significantly raised compared with the NIJD group (PEG, $P = 0{\cdot}006$; C1qSP, $P = 0{\cdot}06$), the discrimination being particularly marked for the PEG complexes (Fig. 3). Compared with age and sex matched normal healthy controls, the levels of CIC were markedly raised in the early RA group (PEG, $P < 0{\cdot}001$; C1qSP, $P < 0{\cdot}001$) but interestingly the amounts in the NIJD group were also raised compared with controls (PEG, $P < 0{\cdot}005$; C1qSP, $P < 0{\cdot}001$). Patients with SLE or TS had less markedly elevated levels of CICs.

Within the early RA group there was an association between the level of RF and the PEG precipitable immune complexes (RAHA, $\rho = 0{\cdot}40$, $P = 0{\cdot}03$; Latex, $\rho = 0{\cdot}48$, $P = 0{\cdot}01$) and there was some association between IgG anti-globulins specific for human IgG and the PEG immune complexes ($\rho = 0{\cdot}35$, $P = 0{\cdot}046$). The IgG anti-globulins specific for rabbit IgG did not show any correlation with the PEG precipitable complexes.

Interestingly, some correlation was found between the levels of PEG precipitable complex and the anti-RANA titre ($\rho = 0{\cdot}33$, $P = 0{\cdot}05$). Further, there was a particularly strong relationship between the amounts of CRP and the PEG immune complexes ($\rho = 0{\cdot}63$, $P = 0{\cdot}004$) in the early RA group. There was also a significant association with the ESR ($\rho = 0{\cdot}46$, $P = 0{\cdot}02$). Whereas, although some of the NIJD group had elevated levels of CRP and raised ESR (Fig. 4) there was no association with the complexes in this group (CRP, $\rho = 0{\cdot}17$, $P = 0{\cdot}19$; ESR, $\rho = 0{\cdot}13$, $P = 0{\cdot}21$).

C1q binding complexes showed little relationship with any of the serological parameters examined. There was no correlation with the complexes precipitated by PEG ($\rho = -0{\cdot}06$) or with the anti-RANA titre ($\rho = -0{\cdot}26$). What little association there was with the anti-globulins was in

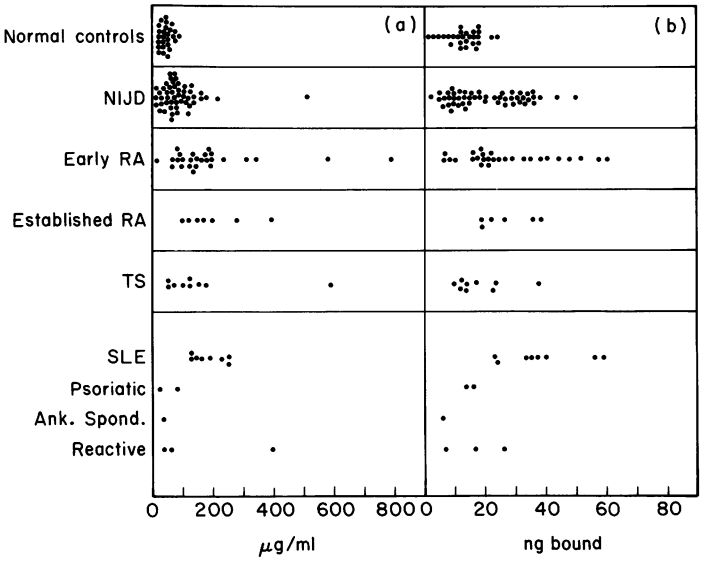

Fig. 3. CICs determined by (a) PEG and (b) C1q solid phase (C1qSP) tests in patients with 'early' joint pains.

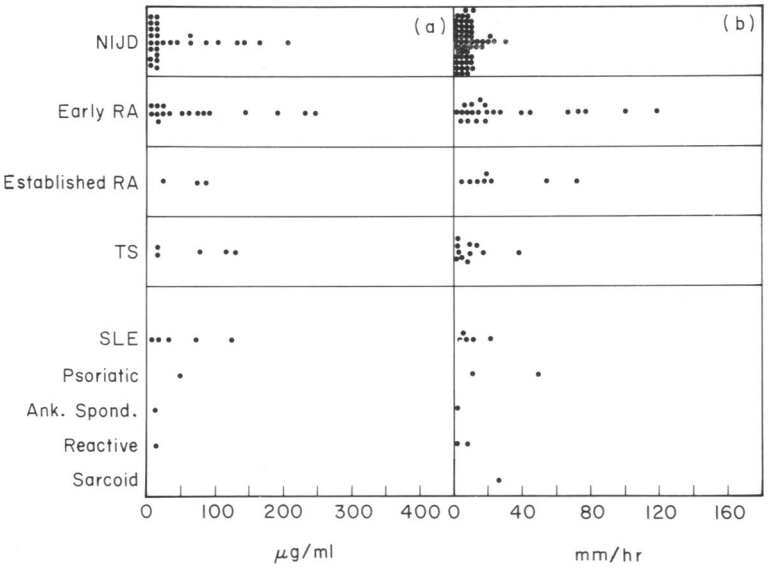

Fig. 4. (a) C-reactive protein (CRP) and (b) erythrocyte sedimentation rate (ESR) in patients with 'early' joint pains.

the negative direction for RAHA ($\rho = -0{\cdot}24$), IgG anti-globulins reacting with rabbit IgG ($\rho = -0{\cdot}37$, $P = 0{\cdot}036$) and for IgG anti-globulins reacting with human IgG ($\rho = -0{\cdot}31$).

Multivariate analysis

Multivariate analysis was carried out on the laboratory data from patients with early RA in comparison with results from those patients diagnosed as having NIJD. The patients were re-classified taking into account radiological and laboratory data, clinical criteria, 1 year after entering the study. Although RF was one of the parameters used in the ARA classification, nevertheless we felt it would be of interest to see to what extent, laboratory findings at presentation, including RF, could help to predict the clinical status of the patient after 1 year. In classical and definite RA not all patients have positive RF and even when they do, this may not be present at the first visit. The analysis was performed on the laboratory data obtained from the first serum sample collected from each patient. We have attempted to obtain a linear discriminant function to enable us to differentiate between the two groups. A stepwise selection method, based on Rao's V, a generalized distance measure (Klecka, 1975) was used. At each stage in the process the variable selected was the one which contributed the largest increase in V when added to the previous variables. This amounts to the greatest overall separation of the groups. At the first stage the Rao's

Table 1. Discriminating power of each test considered alone as measured by Rao's V

Variable	Rao's V
Latex RF	32·9
RAHA RF	31·8
PEG immune complexes	22·3
ESR	17·9
IgG anti-rabbit IgG	16·6
IgG anti-human IgG	15·6
RANA	12·9
C1q immune complexes	1·8

Table 2. Classification assessment of discriminant analysis based on laboratory data obtained from the first serum sample

		Predicted group	
		RA	NIJD
Actual group assessed at 1 year	RA	17	3
	NIJD	2	36

Percentage cases correctly classified = 91%; Chi squared = 37·8; $P = 0{\cdot}0000018$.

V for each separate variable is given, so that the individual discriminant power of each variable may be assessed (Table 1).

The conventional agglutination tests showed the greatest discriminating power with little to choose between them. Both tests were highly specific (100% for latex, 97% for RAHA) and had reasonable sensitivity (60% for latex, 65% for RAHA). In other words, 100% of patients with a positive latex or 97% with RAHA fell in the definite and classical RA group, while 60% of the RA group gave a positive latex test, and 65%, RAHA. The PEG immune complex assay had the same sensitivity as the latex test but was less specific (84%) as might be expected as other conditions besides RA could lead to immune complex formation. All the other assays were relatively poor at discriminating between the two groups when considered on their own.

A number of permutations of tests were examined to see if increased discrimination could be obtained with a combination of results. If all the variables were considered together only four of them were found to contribute useful discriminating information; the PEG immune complex assay, the RAHA test for RF, the ESR and the RANA test. These together gave a high Rao's V (78·2) and the greatest sensitivity (85%) while retaining a good degree of specificity (95%) (Table 2). The RANA test may be omitted with only a small drop in sensitivity to 81% and no loss in specificity at

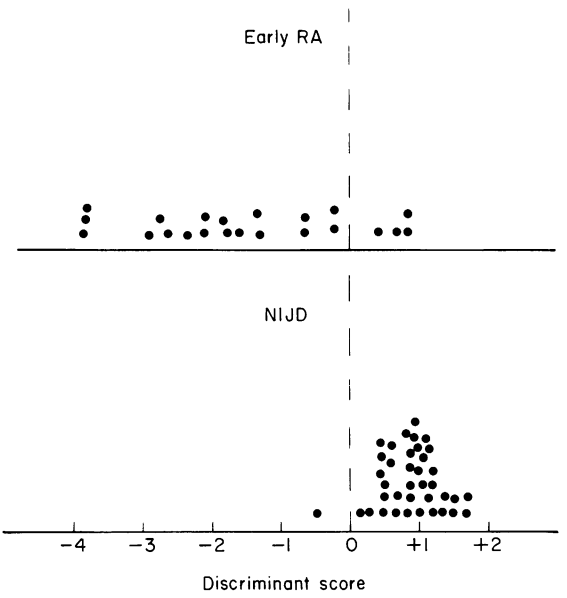

Fig. 5. Discriminant function values based on laboratory data, for two groups of patients with RA and NIJD.

all. If instead, the immune complex assay is left out the loss in sensitivity is greater, falling to 76%. The latex test can easily be substituted for RAHA and gives closely comparable results. The final discriminant function can be expressed as:

$$z = -[0{\cdot}65(\text{RAHA}) + 0{\cdot}52(\text{ESR}) + 0{\cdot}41(\text{PEGCIC}) + 0{\cdot}30(\text{RANA})]$$

where the variables are normalized so that co-efficients provide a measure of the relative importance of each parameter. The distribution of z values for the two groups is shown in Fig. 5.

DISCUSSION

We set out to see whether any one of the immunological parameters, anti-globulins, anti-RANA and CICs, preceded the others chronologically during the development of early RA. Unfortunately, although we were able to investigate patients within a week or so of their first consultation with a general practitioner, careful questioning elicited a history of several weeks or longer since the patient initially became aware of joint symptoms, presumably because these were not usually felt to be serious enough at that stage to warrant a visit to the doctor.

Within these limitations, we have previously reported that titres of anti-RANA are already elevated in early RA to an extent comparable to that observed in long standing disease. We now find that both IgM and IgG RFs which are characteristic features of established RA (Hay *et al.*, 1975) are also present from the earliest possible time since the onset of disease that we have been able to examine the patient. Thus at the earliest stages, significantly elevated levels of agglutinating, presumably mainly IgM, and radioassay detectable IgG antiglobulins are circulating in RA patients. The anti-globulins have specificity for both human and rabbit IgG from the outset as they were detected by agglutination of particles coated with either human or rabbit IgG and by radioimmunoassay against human IgG Fc or rabbit IgG. No attempt was made to dissociate the IgG complexes to reveal hidden IgG RFs which were presumably self-associated (Natvig & Munthe, 1975; Pope *et al.*, 1975), so that these levels are likely to be underestimates.

Immune complexes were also raised early in the disease with a tendency for levels to increase and then decline during the first year. This showed some parallels with the disease activity but, of course, both may reflect a response to treatment. Within the early RA group there was a significant correlation between the levels of complex and anti-globulin indicating that the complexes may well be, at least, partly made up of self-associated antiglobulin. On the other hand, in patients with SLE or TS, although there was some elevation of immune complex levels, the anti-globulins were in the normal range and below the amounts in the NIJD group, indicating that the complexes were likely to be composed of factors other than anti-globulins. Anti-RANA antibody must also be considered as a potential component of the early RA complexes since the titres correlated as strongly with the PEG complexes, as the antiglobulins were.

The lack of correlation between the two immune complex assays suggest that they are recognising different entities. Exactly what the C1q binding complexes are composed of is unclear since the amounts of these complexes were not associated with any of the other factors studied. Jones *et al.* (1981, 1982) also found evidence for two types of immune complex circulating in rheumatoid patients, one involving binding to platelets which correlated poorly with RF levels, as did the complexes detected by our C1q solid phase assay; the other type bound radiolabelled C1q in the presence of polyethylene glycol resembling the complexes detected in our PEG assay and these did correlate with RF.

The relationship between PEG complexes and CRP levels in the early RA patients is interesting as it indicates that these complexes might be involved in the inflammatory process. These complexes are unlikely to contain CRP itself as it has recently been shown that CRP is not a component of immune complexes in inflammatory disease.

Clearly complexes circulate in some forms of NIJD as well as in RA. Perhaps many patients in both the rheumatoid and non-inflammatory groups are responding to the same environmental trigger but only in those with an appropriate genetic background does the disease become chronic. It could be worthwhile to follow-up these patients in the NIJD group who have evidence of raised complexes or anti-globulins to see whether they develop inflammatory arthritis.

In summary, it appears that all the immunological abnormalities typically found in established RA, are present at the earliest phase of the disease which we were able to investigate. Therefore it is not possible to argue in favour of any of the competing hypotheses for the aetiology of RA outlined in the introduction. However, we were able to explore the possibility that the data we had obtained might be of diagnostic value. Multivariate discriminant analysis was used to examine whether we could predict solely on laboratory criteria which of those patients attending a general practitioner clinic with joint pains for the first time would be clinically diagnosed as definite or classical RA at the end of 1 year, as compared with those patients following a non-inflammatory course. Discriminant analysis has mainly been used in business and marketing to aid in decision making, but its use in medicine is increasing and recently it has proved of value in rheumatology in aiding in the selection of criteria for determining clinical remission in RA (Pinals, Masi & Larsen, 1981). Perhaps not surprisingly the conventional agglutination tests for RF were found to give the most discrimination. The patients who are going to develop classical or definite RA already appear to have detectable serological abnormalities at their first visit to the clinic since using a combination of four tests, the RAHA test for RF, the PEG immune complex assay, the ESR and the RANA test it was possible to discriminate accurately between the rheumatoid and non-inflammatory groups, with high sensitivity (85%) and specificity (95%).

We thank Mr Brian Newman and Miss Sigrid Fuhr of the Computer Services Department, Middlesex Hospital Medical School, for advice on the statistical analyses. We are grateful to the Arthritis and Rheumatism Council and the Medical Research Council for financial support.

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