Immunohistological analysis of the synovial membrane: search for predictors of the clinical course in rheumatoid arthritis

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Abstract

Immunohistological features which might predict the clinical course and outcome of rheumatoid arthritis were sought by examining multiple synovial membrane samples obtained by needle biopsy from the knee joints of 57 patients who had not received disease modifying antirheumatic drugs. Clinical measurements, but not biopsies, were repeated one year and three years after starting treatment. A correlation between both the intensity of synovial lining layer thickening and mononuclear cell infiltration and the clinical status at the time of biopsy was seen. After three years of treatment the correlations were maintained in patients who had presented and persisted with milder disease but not in patients who had presented with more active disease.

The rate and extent of disease progression in rheumatoid arthritis is variable, the long term outcome always uncertain,[1] and the response to accepted treatments unpredictable.[2] Several studies have attempted to identify reliable indicators of the clinical course and outcome. Thus it has been suggested that a progressive clinical course and poor outcome is associated with an insidious onset,[3] especially in younger women,[4] with the presence of nodules,[4 5] serum IgM rheumatoid factor,[4 5] and thrombocytosis.[6]

The histopathological changes in rheumatoid synovial membrane range in severity from minimal to intense lining layer thickening, mononuclear cell infiltration, vascular proliferation, and fibrosis. Systematic study of synovial membrane biopsy specimens seeking predictors of outcome has not been undertaken because of reports emphasising difficulties with obtaining representative tissue samples caused by the degree of histological variation within an individual joint.[7–13] These studies, however, included tissue samples obtained from a variety of clinical categories, often after many years of disease, where multiple therapeutic and mechanical factors had been operating. In a series of recent studies of synovial membrane biopsy specimens obtained only from patients with previously untreated disease it was shown that representative tissue could be obtained in most,[14] that the immunohistological features correlated with clinical and laboratory measures of disease activity,[15 16] and that changes in the clinical course of rheumatoid arthritis over one year were reflected by changes in the degree of lymphocyte infiltration found in serial biopsy specimens.[17]

The present study aimed at seeking predictors of outcome from the immunohistological appearances present in synovial membrane biopsy specimens obtained from a larger cohort of patients with rheumatoid arthritis before the introduction of disease modifying treatment. Thus a correlation between the intensity of any pretreatment immunohistological feature and the clinical status at follow up would identify an immunohistological predictor of the clinical course. Fifty seven patients were studied. The immunohistological features were examined in detail to identify characteristics associated with disease outcome as the clinical course evolved during a three year period of follow up. Positive correlations between several of the immunohistological features and clinical measurements were noted on entry to the study. The intensity of synovial lining layer thickening and T cell infiltration correlated positively with the clinical status at follow up in patients who presented with mild disease.

Patients and methods

PATIENTS

Patients presenting with classical or definite rheumatoid arthritis[18] to the department of rheumatology, St Vincent's Hospital, Dublin, between 1984 and 1987 were selected for the study. Patients who had previously received gold salts, D-penicillamine, antimalarial compounds, sulphasalazine, immunosuppressive agents, or oral or intra-articular corticosteroids were excluded. Thus the only antirheumatic treatment the patients had ever received was with non-steroidal anti-inflammatory drugs. The study was approved by St Vincent's Hospital ethics committee, and all patients gave informed consent for synovial biopsy.

CLINICAL AND LABORATORY ASSESSMENTS

Patients were assessed by one of two doctors (MS or MR) at study entry. All were assessed by the same doctor (MS) about one and three years later. Routine indices of disease activity were measured, including Ritchie articular index,[19] duration of morning stiffness, and visual analogue pain score. Grip strength was assessed with a folded sphygmomanometer cuff inflated to 20 mmHg. Routine laboratory assessment included full blood count and Westergren erythrocyte sedimentation rate (mm in first hour). From these measurements a composite index of disease activity (IDA) was calculated, as previously described.[15 17]

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SYNOVIAL BIOPSY

Needle biopsy of one knee joint was carried out with a Williamson-Holt synovial biopsy needle (Shrimpton and Fletcher, Needle Industries, Redditch, Worcestershire, England) under sterile conditions. Local anaesthetic—2% lignocaine—was used. Sterile saline (20 ml) was instilled into the joint to facilitate the biopsy procedure. Multiple synovial samples were obtained from the patellofemoral compartment of the knee joint from as wide a range as possible within reach of the needle. Half the samples were fixed in formalin for paraffin embedding and evaluation by haematoxylin and eosin staining. The remainder were snap frozen, after orientation in optimal cutting temperature compound, in isopentane using liquid nitrogen within 30 minutes of the biopsy procedure. Samples were stored at −70°C until sectioned for immunohistological staining.

HISTOLOGICAL ANALYSIS

Samples fixed in formalin were sectioned to 7 μm and stained with haematoxylin and eosin. The features in all sections were scored on a scale from 0 (normal) to 3 (greatest degree of abnormality). Synovial lining layer hyperplasia was assessed by the cell depth: a cell depth of 1 to 2 was assigned a score of 0, of 3 to 4 a score of 1, of 5 to 7 a score of 2, and a cell depth of greater than 7 a score of 3. A mean score was derived from all of the sections scored from each patient. Mononuclear cell infiltration was assessed on the basis of percentage mononuclear cells identifiable per high power field: thus 5% or less mononuclear cells/high power field was assigned a score of 0, 6 to 29% a score of 1, 30 to 59% a score of 2, and 60% or more a score of 3. Evaluation of the mononuclear cell infiltration was carried out on frozen tissue sectioned to 7 μm. The monoclonal antibodies used were anti-Leu 1 (which identified CD5+ on all T cells), anti-Leu 2a (CD8+ suppressor T cells), anti-Leu 3a (CD4+ helper T cells), and anti-Leu 14 (CD22+ B cells) (Becton Dickinson, Mountain View, Cal 94039, USA). The intensity of the T cell infiltrate was scored on a scale from 0 (<5% of higher power field) to 3 (>60% of high power field); the suppressor and helper cell subsets were expressed as a percentage of the total T cell score and helper to suppressor ratios derived from these scores. The presence or absence of B cells was noted.

STATISTICAL METHODS

Results were analysed using the statistical package for the social sciences X. Data were correlated using the Pearson coefficient with Yates's correction; comparative data were analysed with Mann-Whitney, Wilcoxon, and χ^2 coefficients.

Results

IMMUNOHISTOLOGICAL AND CLINICAL FEATURES ON ENTRY TO THE STUDY

Table 1 gives clinical details of the 57 patients included in the study. The mean disease duration was 26 months; only two patients had rheumatoid arthritis for more than four years. In the synovial biopsy specimens measurable lining layer thickening on a scale of 1 to 3 was present in 92% of patients. A mononuclear cell infiltrate was found in 78%. Characteristic focal perivascular lymphoid aggregation was present in biopsy specimens obtained from 12 (21%) patients. Thus in most biopsy specimens the mononuclear cell infiltration was diffuse in appearance. As expected, when monoclonal antibodies were used most mononuclear cells in all specimens were CD5+ T cells. CD4+ helper T cells and CD8+ suppressor/cytotoxic T cells were identified in varying numbers. B cells were identified in biopsy specimens from 34% of patients. Significant correlations were shown between the IDA on entry to the study and the degree of synovial lining layer thickening ($p<0.01$), mononuclear cell infiltration ($p<0.01$), and CD5+ T cell infiltration ($p=0.02$). No correlations were found between the clinical status on entry to the study and numbers or percentages of helper or suppressor/cytotoxic T cells, or with the presence of B cells. Thus the most intense histological change was seen in the synovial tissue obtained from patients with most active clinical features.

Table 1 Clinical and demographic details on entry to study. Results are given as mean (SD) unless otherwise stated

Total patients (n)	57
Total female (No (%))	41 (72)
Mean age (range) years	49 (18–72)
Mean age onset of RA (range) years	47 (18–71)
Mean duration RA (range) months	26 (1–144)
Number rheumatoid factor positive (%)	54 (95)
No with nodules (%)	8 (14)
Ritchie articular index	15·9 (11·6)
Pain, visual analogue scale	4·6 (2·6)
Duration morning stiffness (min)	204 (364)
Grip strength (mmHg)	132 (71)
Haemoglobin (g/l)	120 (12)
Erythrocyte sedimentation rate (mm/h)	55 (30)
Index of disease activity	2·5 (0·6)

IMMUNOHISTOLOGICAL FEATURES ON ENTRY AND CLINICAL FEATURES AT ONE YEAR

Forty nine patients were re-evaluated clinically after one year. All were receiving drug regimens considered by their rheumatologists to be appropriate: seven non-steroidal anti-inflammatory drugs alone, seven antimalarial compounds, 27 sodium aurothiomalate, and seven methotrexate. Thirteen were receiving corticosteroids (prednisone 5–10 mg/d) and, of these, two were receiving low dose corticosteroids alone. Most patients showed measurable clinical improvement. Thus the mean IDA had fallen from 2·5 to 1·8 on a scale of 1 to 4.

The immunohistological features seen on entry to the study were correlated with the clinical status at one year's follow up. Adequate tissue for analysis of lining layer thickening was available in 44 biopsy specimens and for mononuclear cell infiltration in 47. A significant positive correlation was seen between the intensity of synovial lining layer thickening and the IDA ($p=0.01$) (table 2). No correlations were found between the intensity of mononuclear cell or of CD5+, CD4+, or CD8+ T

Table 2 Correlations between immunohistological features and clinical status at follow up

Immunohistological feature	*n*	*r*	*p Value*
At one year's follow up:			
Lining layer thickness	44	0·35	0·01
T cell infiltration	47	0·14	0·18
At three years' follow up:			
Lining layer thickness	31	0·27	0·07
T cell infiltration	27	0·26	0·09

cell infiltration, or with the presence of B cells. No correlation between the presence of focal perivascular lymphoid aggregation and the IDA at one year was found. Similar analyses of two individual components of the IDA after one year's follow up were performed with similar results. The Ritchie articular index and the erythrocyte sedimentation rate are often used individually by clinicians to measure disease activity. Synovial lining layer thickening correlated with the erythrocyte sedimentation rate ($p=0·002$) and with the Ritchie articular index ($p=0·02$). Thus the greater the degree of lining layer thickening in the pretreatment biopsy specimen, the greater the degree of disease activity after one year. No positive correlations with mononuclear cell infiltration were seen.

IMMUNOHISTOLOGICAL FEATURES AT ENTRY AND CLINICAL FEATURES AT THREE YEARS

Thirty one patients were evaluated after three years' follow up. Therapeutic adjustments had been made as considered appropriate. Thus 18 patients were receiving gold salts, five methotrexate, three antimalarial compounds, seven low dose corticosteroids and one a non-steroidal anti-inflammatory agent only. The overall initial clinical improvement had been maintained with a mean IDA value of 1·9. The previously noted correlation between the intensity of synovial lining layer thickening in the pretreatment biopsy specimens and the clinical status was not present after three years (table 2). No correlations were found between the intensity of mononuclear cell or of CD5+, CD4+, or CD8+ T cell infiltration, or with the presence of B cells.

EFFECTS OF DISEASE ACTIVITY AT THE TIME OF BIOPSY ON CLINICAL CORRELATIONS AT ONE AND THREE YEARS

Further analyses were performed to determine the possible effects of disease activity at the time of entry to the study on the relation between the immunohistological features and the clinical course. The midpoint on the IDA scale of 1 to 4 was selected to divide patients into those presenting with milder disease (IDA 1 to 2·5) or more active disease (IDA 2·6 to 4). At one year's follow up the mean IDA value of patients presenting with IDA $\leq 2·5$ had diminished by only 13%; the mean value of patients presenting with IDA $>2·5$ had diminished by 64%. The previously noted positive correlation between synovial lining layer thickness in the pretreatment biopsy specimen and the clinical status at one year's follow up was present only in patients presenting with IDA $\geq 2·5$ ($p=0·02$) (table 3). Similarly, at three years' follow up the mean IDA values of patients presenting with IDA $\leq 2·5$ and $>2·5$ had diminished by 15% and 60% respectively. A more striking correlation between lining layer thickness and clinical status emerged in those patients presenting with IDA $\leq 2·5$ ($p=0·009$). Moreover, a positive correlation also emerged between the intensity of CD5+ T cell infiltration and clinical status in patients presenting with IDA $\leq 2·5$ ($p=0·002$). Thus in patients presenting for synovial biopsy with milder disease, but not in patients presenting with more active disease, the intensity of some immunohistological features continued to correlate with the clinical status after three years' follow up.

Table 3 Effect of disease activity at the time of entry to the study on the correlation between immunohistological features and clinical status at follow up

Immunohistological feature	*Disease activity (IDA) on entry to study*					
	$\leq 2·5$			$>2·5$		
	n	*r*	*p*	*n*	*r*	*p*
At one year's follow up:						
Lining layer thickness	24	0·44	0·02	20	0·35	0·07
T cell infiltration	27	0·13	0·26	20	0·18	0·22
At three years' follow up:						
Lining layer thickness	11	0·69	0·009	20	0·13	0·29
T cell infiltration	10	0·82	0·002	17	−0·11	0·33

Discussion

Fifty seven patients with rheumatoid arthritis were assessed clinically and subjected to needle biopsy of the synovial membrane before receiving any specific disease modifying antirheumatic drug treatment. As described previously in a smaller cohort of patients,[15] a correlation between immunohistological and clinical features was confirmed. The patients' clinical status was reassessed after one and three years' follow up. Correlations were sought between the immunohistological features at presentation and subsequent clinical measurements which might retrospectively identify immunohistological predictors of clinical outcome.

After one and three years' follow up clinical improvement was shown, especially in those patients presenting with more active disease. When correlations were sought an association between the intensity of synovial lining layer thickening at presentation and the clinical status at one year was found. Further analysis showed that this correlation existed only among patients presenting with mild disease (IDA $\leq 2·5$). At three years' follow up the correlation between the immunohistological features at presentation and clinical status among patients presenting with milder disease was even more significant. No correlation between the immunohistological features at presentation and clinical status at follow up was found among patients presenting with more active disease (IDA $>2·5$). Thus in a subgroup of patients presenting with an IDA of $\leq 2·5$ a synovial biopsy provided an indication of clinical status at three years. The explanation for this observation is likely to be that fewer patients presenting with milder disease had received specific disease modifying antirheumatic drugs. The clinical course of this

milder subgroup changed little, so that the initial correlations between the immunohistological features and the clinical status at presentation persisted. The patients presenting with more active disease were more likely to have received disease modifying drugs which reduced indices of disease activity, causing a reversal of correlations initially seen. To resolve fully the question asked in this study it would be necessary to analyse the disease course and outcome in patients with rheumatoid arthritis from whom disease modifying drugs had been withheld over an extended period of follow up. This, of course, would be unacceptable.

In rheumatoid arthritis it is generally accepted that T and B cells pass through synovial venules to form lymphocyte-rich perivascular infiltrates[20] containing predominantly CD4+ T helper cells and HDA-DR+ mononuclear cells.[21–26] T cells may migrate through the synovium and occupy transitional areas[27] containing a mixture of other cell types, including interdigitating dendritic cells, macrophages, plasma cells, and fibroblasts.[28] Most cells occupying the thickened lining layer are also mononuclear phagocytic cells which migrated from the synovial venules.[29–33] It has been shown previously that the intensity and composition of the lymphocyte infiltration in the sublining layer of the synovial membrane changes according to the clinical course.[17] In this study the clinical course which evolved over three years was influenced by the initial degree of lymphocyte infiltration in patients presenting with milder disease. In patients presenting with more active disease, likely to receive disease modifying treatment at an earlier stage, neither the presence of characteristic focal lymphoid aggregation nor more intense mononuclear cell infiltration at presentation predicted the clinical course. It is entirely possible, however, that a more aggressive clinical course would have followed if disease modifying drugs had been withheld from this subgroup. It has not yet been determined whether the intensity of lining layer thickening is altered during the clinical course and by the response to treatment.

Since the start of this study, when the immunohistological examinations were performed, advances in the methodology for analysis of cell subpopulations residing in the synovial membrane have been made. Thus the scope for further prospective studies seeking more precise immunohistological predictors of outcome is greater.

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