Histology of the synovial tissue: Value of semiquantitative analysis for the prediction of joint erosions in rheumatoid arthritis

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Abstract

Objective

Routine histologic techniques are still the main procedure in the study of the synovial biopsy. The relationship between the typical histological changes of rheumatoid synovium and clinical manifestations has not been studied in detail.

Methods

With the aim of determining whether a simple semiquantitative method of evaluating the changes in closed synovial biopsies was of clinical value in assessing both the diagnosis and prognosis of rheumatoid arthritis (RA) patients, we evaluated retrospectively 72 synovial biopsy specimens (26 RA patients, 30 patients with other inflammatory diseases and 16 osteoarthritis patients). Scores (0-10) were assigned to each biopsy specimen for each of 6 histologic features: synoviocyte hyperplasia; fibrosis in the subsynovial layer; proliferating blood vessels; perivascular infiltrates of lymphocytes; focal aggregates of lymphocytes; and diffuse infiltrates of lymphocytes. Scores were compared between the 3 groups and also between the RA subgroups with early and late disease; positive and negative rheumatoid factor; with and without joint erosions; and with and without systemic disease.

Results

Significant differences in the mean global score (mean of the 6 scores) were found both between RA and osteoarthritis and between other inflammatory diseases and osteoarthritis ($p < 0.01$). The mean global score for RA was higher than the mean global score obtained for the other inflammatory diseases, but the difference was not significant. We found a significantly higher mean global score in the RA patients with erosions in comparison to the RA patients without erosions, this difference being particularly evident for the lymphocyte perivascular infiltrate ($p < 0.05$). There were no significant differences between the other RA subgroups.

Conclusion

In this study we have identified differences, using routine histologic techniques, between the rheumatoid synovial membrane of patients with and without erosions. Based on our present observations we suggest that the intensity of inflammatory histological features and, in particular, a high percentage of vessels with perivascular lymphocyte infiltrate might be of prognostic value in RA.

Key words

Rheumatoid arthritis, synovial tissue, diagnosis, prognosis, erosions.

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Introduction

Routine histologic techniques are still the main procedure in the study of rheumatoid synovium. An extensive effort has been made to characterise the most relevant histologic features of joint inflammation. In fact, a variety of aspects have been described in the rheumatoid synovium: proliferation of the synovial lining layer, infiltration by lymphocytes and plasma cells, increased vascularity and fibrosis (1). Despite the occurrence of considerable overlap with other inflammatory diseases (2), these histological features are generally more severe in rheumatoid disease than in other rheumatic inflammatory disorders and might be correlated with the clinical activity of joint disease, as proposed by Gallagher *et al*. (3), although these data have not effectively increased the precision with which pathologists can establish the diagnosis or the prognosis of rheumatoid arthritis (RA) (4, 5).

Considering these previous results, we have tried to re-analyse this relevant issue through the evaluation of 72 synovial biopsy specimens (26 RA patients, 30 patients with other inflammatory joint diseases and 16 osteoarthritis patients). We adapted a scale proposed by Rooney *et al*. (6) to analyse the slides reviewed. Scores (0-10) were assigned to each biopsy specimen for each of 6 histological features: synoviocyte hyperplasia; fibrosis in the subsynovial layer; proliferating blood vessels; perivascular infiltrate of lymphocytes; focal aggregates of lymphocytes; and diffuse infiltrate of lymphocytes. Subsequently, scores were compared between the 3 groups and also between the following RA subgroups: early and late disease; positive and negative rheumatoid factor; with and without joint erosions; with and without systemic disease. Interestingly, we found a significantly higher mean global score in the RA patients with erosions in comparison with the RA patients without erosions, this difference being particularly evident for the intensity of the lymphocyte perivascular infiltrate ($p < 0.05$).

Material and methods

This study included all patients who underwent needle biopsy (7) or arthroscopic examination with biopsy between 1993 and 1997, with a minimum follow-up (after the biopsy) of one year at the Rheumatology Unit of Santa Maria Hospital (Lisbon). During this period of time 238 synovial biopsies were done (72% female, mean age 47.6 ± 18.6 years), but after review of the clinical records only 105 (71.4% female, mean age 49.4 ± 18.1 years) had a minimum follow-up of one year. Most of the excluded patients had been referred for biopsy from other rheumatology units. In addition, 33 cases were excluded due to an insufficient amount of synovial tissue (less than 8 mm^2). The 72 patients finally included in the study had a mean age of 48.6±19.4 years, 50 (69.4%) were female and 22 (30.6%) were male. Multiple synovial samples were obtained from the knee suprapatellar pouch (51), knee arthroscopy (7), ankle (4), wrist (3), hip (2), elbow (2), proximal interphalanges (2), and shoulder (1).

For every patient the following data were collected from the clinical records: disease duration; duration of inflammatory symptoms in the joint where the biopsy was taken; Westergren erythrocyte sedimentation rate (ESR) at the time of biopsy; the diagnostic hypothesis before the biopsy; the pathologist's diagnosis and the final diagnosis.

Patients were considered to have RA if they satisfied the American College of Rheumatology (ACR) RA criteria (8). All cases were evaluated according to a protocol that included the assessment of extra-articular involvement, presence of Sjögren's syndrome (SS), rheumatoid factor detection, presence of erosive disease, disease duration and patient functional class (ACR criteria, ref. 9). Patients were considered to have extra-articular manifestations if at least one of the following clinical features could be detected: subcutaneous nodules; pulmonary fibrosis confirmed by chest roentgenograms and lung function tests; echocardiographic evidence of pericardial effusion or pleural effusion shown by chest roentgenograms; Felty's syndrome ($< 2 \times 10^9$/l granulocytes and splenomegaly); cutaneous vasculitis (leukocytoclastic vasculitis histologically proven); and/or noncompressive neuropathy confirmed by electromyography. The diagnosis of SS was based on the clinical

symptoms of dry eyes and dry mouth, confirmed by a positive Schirmer's test and/or keratoconjunctivitis sicca, with involvement of salivary glands documented by positive lip biopsy and/or salivary scintigraphy. Patients were considered seropositive if the Waaler Rose test showed a titer > 1:64 on 2 or more occasions.

Specimens were fixed in Bouin's fluid (10), embedded in paraffin, cut into sections 6 to 10 μm thick and stained with hematoxylin and eosin. Synovial sections were coded and randomly analysed by a blinded observer (JEF). The samples were examined under 400x magnification and photographed with an Olympus BH-2 microscope. The surface area was estimated using an eyepiece with a squared graticule and diameters were measured using a micrometer eyepiece. For each patient 3 to 6 samples with a mean area of 58.3 ± 37.1 mm^2 were studied. This area exceeds the minimum area of rheumatoid synovium needed to allow accurate microscopic analysis of synovial inflammation (11). The reproducibility of the results was confirmed by the random and blinded re-observation of one in each 10 slides.

Six histological features were scored on a scale of 0-10 (6, 12):

- The mean number of synoviocytes in the lining layer, with a possible number of layers ranging from 1 (score 0) to more than 10 (score 10).
- The mean percentage of fibrosis beneath the synovial layer. As the percentage of fibrosis in the section increased, the score increased: less than 10% (score 0), 10-15% (1), 15-20% (2), 20-25% (3), 25-30% (4), 30-40% (5), 40-50% (6), 50-60% (7), 60-70% (8), 70-80% (9), more than 80% (10).
- The mean density of blood vessels per high power field (under 400x magnification). As the number of vessels increased, the score increased: less than 3 (score 0), 4-5 (1), 6-7 (2), 8-9 (3), 10-11 (4), 12-13 (5), 14-15 (6), 16-17 (7), 18-19 (8), 20-22 (9), more than 22 (10).
- The mean percentage of vessels with perivascular infiltrates of lymphocytes. Perivascular infiltrates were characterised as aggregates of lymphocytes that were contiguous with the vessel wall and were no more than 10 cells in diameter: less than 5% (score 0), 5-10% (1), 10-20% (2), 20-30% (3), 30-40% (4), 40-50% (5), 50-60% (6), 60-70% (7), 70-80% (8), 80-90% (9), 100% (10).
- The mean number of lymphocytes in the diameter of the focal aggregates of lymphocytes. This feature also assessed perivascular infiltrates that were more than 10 cells in diameter. As the cell numbers in the diameter of the aggregates increased, the score for the section increased: less than 11 (score 0), 11-15 (1), 15-20 (2), 20-25 (3), 25-30 (4), 30-35 (5), 35-40 (6), 40-45 (7), 45-50 (8), 50-55 (9), more than 55 (10).
- The mean percentage of lymphocytes per high power field (under 400x magnification). This feature assessed only the lymphocytes that did not fall into either of the above categories of perivascular or focal aggregates. An increasing percentage of lymphocytes correlated with a higher score: 0 (score 0), 1-10% (1), 10-20% (2), 20-30% (3), 30-40% (4), 40-50% (5), 50-60% (6), 60-70% (7), 70-80% (8), 80-90% (9), 90-100% (10).

Statistical analysis was performed using the Student t-test.

Results

Characterisation of the study population

The 72 patients were divided into three groups based on their diagnosis at the end of the follow-up period: 26 RA patients, 30 patients with other inflammatory disorders and 16 osteoarthritis (OA) patients (Table I).

Regarding the 26 RA patients, 4 were male (15.4%) and 22 female (84.6%), the mean age was 55.5±12.7 years, the mean disease duration was 8.1 ± 7.1 years and the mean duration of the inflammatory symptoms in the joint where the biopsy was taken was 5.4 ± 5.6 years. At the time of the biopsy the mean Westergren ESR was 46.2 ± 28.6 mm/1st hr. Rheumatoid factor was detected in the serum of 18 (69.2%) patients, radiological bone erosions were visualised in 15 (57.7%)

Table I. Characterisation of the study population.

	Patients with rheumatoid arthritis (n = 26)	Patients with other inflammatory joint diseases (n = 30)	Patients with osteoarthritis (n = 16)
Male/female (no. and %)	4 (15.4%)/ 22 (84.6%)	16 (53.3%)/ 14 (46.7%)	4 (25%)/ 12 (75%)
Mean age ± SD (m-M), yrs.	55.5 ± 12.7 (32 - 78)	35.2 ± 19.5 (7 - 74)	61.6 ± 14 (23 - 77)
Disease duration ± SD (m-M), yrs.	8.1 ± 7.1 (0 - 22)	2.8 ± 3.1 (0.1 - 11)	3.8 ± 3.3 (0 - 10)
Synovitis duration ± SD (m-M), yrs.	5.4 ± 5.6 (0-20)	2.4 ± 3.1 (0.1 - 11)	3 ± 3.5 (0 - 10)
ESR ± SD (m-M) mm/1st hr.	46.2 ± 28.6 (5 - 105)	27.3 ± 28.8 (2 - 105)	21.9 ± 19.7 (1 - 59)
Rheumatoid factors (no. and %)	18 (69.2%)	—	—
Bone erosions (no. and %)	15 (57.7%)	—	—
Systemic manifestations (no. and %)	6 (23.1%)	—	—
Sjögren's syndrome (no. and %)	3 (11.5%)	—	—
Class ACR I (no. and %)	7 (30%)	—	—
Class ACR II (no. and %)	16 (61.5%)	—	—
Class ACR III (no. and %)	1 (3.8%)	—	—
Class ACR IV (no. and %)	2 (7.7%)	—	—

patients, 6 (23.1%) patients had systemic manifestations [pulmonary fibrosis in 2, rheumatoid nodules in 2, rheumatoid nodules and pulmonary fibrosis in 1, rheumatoid nodules and large granular lymphocytes (LGL) in 1], and 3 (11.5%) patients had associated SS. From a functional point of view the RA patients could be classified as follows: 7 (30%) in class I, 16 (61.5%) in class II, 1 (3.8%) in class III (3.8%) and 2 (7.7%) in class IV. At the moment of the biopsy all patients were receiving non-steroidal anti-inflammatory drugs, 10 were taking slow acting anti-rheumatic drugs (methotrexate 2, gold 5, antimalarials 3) and 19 were on low-dose prednisone. The characteristics of the RA population studied were consistent with the disease pattern of RA previously described in Portugal (13, 14).

Thirty cases were classified as "other inflammatory diseases". Sixteen were male (53.3%) and 14 were female (46.7%), with a mean age of 35.2 ± 19.5 years, a disease duration of 2.8 ± 3.1 years and a mean duration of inflammatory symptoms in the joint where the biopsy was taken of 2.4 ± 3.1 years. At the time of the biopsy the mean Westergren ESR was 27.3 ± 28.8 mm/1st hr. This group included 6 cases of ankylosing spondylitis [AS, modified New York Criteria (15)], 3 psoriatic arthritis (PA, Wright and Moll criteria (16)], 4 juvenile idiopathic arthritis (JIA, revision of the proposed criteria for juvenile idiopathic arthritis, Durban 1997 (17)], 2 undifferentiated connective tissue diseases (i.e., patients with incomplete features of the classic connective tissue diseases (18)], 1 systemic lupus erythematosus (SLE, 1982 ACR revised criteria (19)], 3 crystal associated synovitis (1 case of calcium pyrophosphate dehydrate crystals identified in the synovial fluid and 2 cases of monosodium urate monohydrate crystals identified in the synovial fluid), 1 haemochromatosis (excess iron deposits demonstrated in liver biopsy), 1 palindromic rheumatism (recurrent, short-lived, attacks of painful swelling joints without articular damage (20)], 8 undiagnosed monoarthritis and 1 undiagnosed polyarthritis (after a mean follow-up of 2.2 years; minimum 1 year and maximum 4 years).

Sixteen patients were classified as having knee OA (ACR diagnostic criteria) (21); 4 of these were male (25%) and 12 female (75%). The mean age was 61.6 ± 14 years, the mean disease duration was 3.8 ± 3.3 years and the mean duration of inflammatory symptoms in the joint from which the biopsy was taken was 3 ± 3.5. At the time of the biopsy the mean Westergren ESR was 21.9 ± 19.7 mm/1st hr.

Correlation of histological features with the final clinical diagnosis

Significant differences for each of the histological scores and for the mean global score (mean of the 6 scores) were found between RA and OA patients (Table II) and between other inflammatory disorders and OA (Table III). In fact, the RA mean global score was 19.8 ± 9.7, which was significantly ($p < 0.01$) higher than the OA mean global score (11.1 ± 7.4). In addition, the mean global score for the other inflammatory disorders (17.7±10.3) was significantly higher than the mean global score of the OA patients (11.1 ± 7.4) ($p < 0.05$).

The mean global score for RA was higher than the mean global score obtained for the other inflammatory diseases, but the difference was not significant. Although RA scores were slightly higher than those observed for the other inflammatory disorders, they did not reach a significant difference.

Correlation of histological features with clinical manifestations of RA

We found a significantly higher mean global score in the RA patients with erosions (23 ± 10.5) in comparison with the RA patients without erosions (14.6 ± 10.0; $p<0.05$; Table IV). This difference was particularly evident for the intensity of the lymphocyte perivascular infiltrate (4.5±3.6 versus 1.9 ± 2.3; $p < 0.05$). Patients with and without erosions were similar concerning the mean age and mean disease duration (56.8 ± 12.8 years and 8.6 ±7.4 years versus 53.6 ± 12.8 years and 7.4 ±7 years). One of the patients with erosive disease had undergone a biopsy one year before the appearance of erosions. The histological features were already similar to those associated with erosive disease (mean global score 37, lymphocyte perivascular infiltrate score 7). Finally, we observed the presence of multinucleated giant cells in 3 patients with erosions and in none of

Table II. Comparison of histological features in the RA and OA patients studied.

Histological scores	RA (n = 26) mean ± SD (m-M)	OA (n = 16) mean ± SD (m-M)	P
Global	19.8 ± 9.7 (4 - 41)	11.1 ± 7.4 (1 - 31)	< 0.01
Lining layer	1.8 ± 1.7 (0-6)	0.6 ± 0.9 (0-3)	< 0.05
% Fibrosis sub-lining layer	7.5 ± 3.2 (0-10)	6.6 ± 3.8 (0-10)	NS
Vessel density	3.3 ± 2 (1-8)	3.1 ± 2.6 (0-9)	NS
% Vessels with infiltrate	3.3 ± 3.3 (0-10)	0.4 ± 1.1 (0-9)	< 0.01
Lymphocyte clusters diameter	1.9 ± 3 (0-10)	0.1 ± 0.5 (0-2)	< 0.05
% Lymphocytes in synovium	2.1 ± 2.1 (0-8)	0.3 ± 0.8 (0-3)	< 0.01

Table III. Comparison of histological features in the patients with other inflammatory diseases (OID) and those with OA.

Histological scores	OID (n=30) mean ± SD (m-M)	OA (n=16) mean ± SD (m-M)	P
Global	17.7 ± 10.3 (0-39)	11.1 ± 7.4 (1-31)	< 0.05
Lining layer	1.5 ± 1.7 (0-6)	0.6 ± 0.9 (0-3)	< 0.05
% Fibrosis sub-lining layer	6.6 ± 3.7 (0-10)	6.6 ± 3.8 (0-10)	NS
Vessels density	3.2 ± 2.3 (0-9)	3.1 ± 2.6 (0-9)	NS
% Vessels with infiltrate	2.5 ± 3 (0-10)	0.4 ± 1.1 (0-9)	< 0.05
Lymphocyte clusters diameter	1.7 ± 2.4 (0-6)	0.1 ± 0.5 (0-2)	< 0.05
% Lymphocytes in synovium	2.3 ± 2.4 (0-8)	0.3 ± 0.8 (0-3)	< 0.01

Table IV. Comparison of histological features in patients with erosive RA and non-erosive RA.

Histological scores	Erosive RA (n = 15) mean ± SD (m-M)	Non-erosive RA (n = 11) mean ± SD (m-M)	P
Global	23 ± 10.5 (12 - 41)	14.6 ± 10.0 (0 - 27)	< 0.05
Lining layer	1.6 ± 1.7 (0 - 6)	1.7 ± 1.7 (0 - 4)	NS
% Fibrosis sub-lining layer	7.7 ± 3.1 (0 - 10)	5.6 ± 4.2 (0 - 10)	NS
Vessel density	3.5 ± 1.9 (1 - 6)	2.7 ± 2.3 (0 - 8)	NS
% Vessels with infiltrate	4.5 ± 3.6 (0 - 10)	1.9 ± 2.3 (0 - 6)	< 0.05
Lymphocyte cluster diameter	2.8 ± 3.6 (0 - 10)	1 ± 2.1 (0 - 6)	NS
% Lymphocytes in synovium	2.5 ± 2.5 (0 - 8)	1.6 ± 1.6 (0 - 4)	NS

the RA patients without erosions.

There were no significant differences between RA patients with and without rheumatoid factors and between RA patients with and without systemic manifestations. The histological scores of the patients with more than one year of synovitis were not significantly different from the scores of the RA patients with early synovitis. As could be expected, there was a positive correlation between the Westergren ESR and the mean global score.

Discussion

Our results suggest that high semiquantitative inflammatory synovial scores are associated with bone erosions, this being particularly evident for the percentage of vessels with lymphocyte infiltrate. In addition, one patient with erosions in our series had undergone a biopsy one year before the appearance of radiological erosions and, interestingly, the histological scores were already similar to those associated with erosive disease.

Fassbender and Gay, after reviewing approximately 14,500 RA synovial samples, have proposed a 3-phase model for rheumatoid joint destruction (22). The first phase is an aggressive and invasive process caused by an homogenous cell mass without inflammatory cells. This phase is presumed to be specific for RA, although it is very brief and thus rarely observed. In fact, we were not able to identify this phase in our samples. The second phase is characterised by a mixed cell infiltrate (migrating lymphocytes, plasma cells and macrophages), followed by maturation of the synovial tissue into a definite collagenous scar (third phase). Most of our observations could be included in Fassbender's second phase. Although their series is one of the largest published, Fassbender and Gay did not correlate their findings with the clinical features of the patients, in particular with the presence or absence of bone erosions.

Recent immunohistochemical studies have shown that bone erosions are associated with a high frequency of macrophages and synoviocytes in the lining layer (23, 24). On the other hand, it has also been proposed that a possible relationship between the intensity of lymphocyte infiltrate and the activity of RA could exist (25, 26), and also that lymphocyte migration (27) and perivascular lymphocyte infiltrates could be early events in RA, being hypothetically already present in asymptomatic joints (28).

Our results and these previous data point to a possible prognostic value of the perivascular lymphocyte infiltrate as an early predictive factor of the future development of bone erosions. The importance of the lymphocyte infiltrate as a prognostic factor in RA might be theoretically linked to the association between RA and the MHC class II allotype, reinforcing the role of lymphocyte-macrophage interactions as mediators of RA pathogenesis.

There were no differences in the frequency of each histological score between rheumatoid factor positive RA and rheumatoid factor negative RA. This is in accordance with the results of Fujinami *et al.* (4) and supports the hypothesis that rheumatoid factor positive patients do not have different inflammatory histological features. Furthermore, several groups have reported a lower prognostic value for the rheumatoid factor than it was previously believed to have. In fact, recently we found a low association between rheumatoid factor and ACR functional class (29) and others have failed to demonstrate a positive correlation between rheumatoid factor and severe disease manifestations (30), radiological erosions (31), or the need for orthopaedic surgery (32). A poor correlation between clinical disease and total serum antibody levels does not imply that rheumatoid factor is a mere bystander in the pathogenesis of RA, however. This inconsistent relationship between antibodies and autoimmune disorder is well known, even in conditions where the role of the autoantibodies is not in doubt, such as in the antiphospholipid syndrome (33).

Histologic studies that have tried to detect specific features of early and chronic RA have been equivocal (34, 35). Schumacher and Kitridou have suggested that early RA synovitis might be characterised by synovial lining hyperplasia, vascular changes and the accumulation of lymphocytes (36). In addition, Konttinen *et al.* have proposed that the most typical findings of chronic RA are large T cell and plasma cell infiltrates (37). However, Tak *et al.* have shown that there were no immunohistochemistry differences between the synovium of 31 RA patients with early RA (less than 1 year) and 35 RA patients with longstanding disease (more than 5 years) (38). This view is sustained by our present results and also by our previous observations in adjuvant arthritis of an inflammatory process coupled with changes in the nerve pattern preceding the joint inflammatory signs (39, 40). Moreover, Kraan *et al.* demonstrated that asymptomatic synovitis precedes clinically manifest arthritis in an animal model and also in RA patients (41). The finding that histological features might not be dependent on the synovitis duration is consistent with the concept that early RA is preceded by a sub-clinical inflammatory process of unknown duration but already detectable by histologic examination.

Two recent reports depicted immunohistological features that might be associated with a more aggressive radiological course (23, 24). In this study we have

identified differences, using routine histologic techniques, between the rheumatoid synovial membrane of patients with and without erosions. Based on our present observations, we suggest that the intensity of inflammatory histological features and, in particular, a high percentage of vessels with perivascular lymphocyte infiltrates might be of prognostic value in RA. These data need to be confirmed by a prospective study.

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