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p53 Expression in rheumatoid and psoriatic arthritis synovial tissue and association with joint damage

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EXTENDED REPORT

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Background: Overexpression and functional mutations of p53 have been found in the synovial tissue (ST) of patients with rheumatoid arthritis (RA), but their clinical significance remains unclear.

Objective: To analyse p53 expression in the ST of patients with RA and psoriatic arthritis (PsA) and its association with joint damage.

Methods: Synovial biopsy specimens were obtained by arthroscopy in 45 patients (27 RA, 18 PsA). Radiographs of hands, feet, and the joint undergoing arthroscopy were obtained to evaluate the presence of erosive disease. Synovial cell populations were analysed using CD4, CD8, CD138, CD20, and CD68 monoclonal antibodies (mAbs). The p53 protein was determined by immunohistology using DO7 mAb in 34 patients (18 RA, 16 PsA). In 11 patients with early RA, the association between p53 and 1 year progression of radiographic damage was analysed using the Larsen-Scott method.

Results: The p53 protein was detected in 16/18 (89%) patients with RA and in 9/16 (56%) patients with PsA, but its expression in RA was significantly higher than in PsA. In RA, p53 expression was significantly associated with erosive disease, and its scores were higher in patients with radiological progression. CD68 expression was also associated with erosions and radiological progression in RA. No association was found between either p53 or CD68 and erosive disease in PsA.

Conclusions: These results suggest that p53 ST overexpression and association with joint damage is characteristic of RA rather than PsA, and that p53 ST expression might be a prognostic marker of joint damage in RA.

Overexpression and functional mutations of the tumour suppressor p53 protein have been demonstrated in rheumatoid arthritis (RA) synovial tissue, most extensively in patients with advanced, destructive disease.¹ Although overexpression of p53 in tissues was originally thought to be a surrogate marker for mutations,² subsequent studies have demonstrated wild-type p53 expression in RA and other inflammatory diseases, including reactive arthritis and inflammatory osteoarthritis.³ Recent studies suggest that p53 induction is a general phenomenon in inflammation, directed at modulating normal inflammatory responses.⁴ Although a higher p53 expression has been demonstrated in synovial tissue (ST) from patients with destructive RA disease, no prospective studies exploring the association between p53 expression and joint damage have been published.

Psoriatic arthritis (PsA) develops in about 5–25% of patients with psoriasis and may be as severe as RA, with almost 20% of patients developing severe, destructive, deforming arthritis.⁵ Although classified as a spondyloarthropathy, PsA shares pathogenic mechanisms with RA, including the Th1 cytokine derived pattern,⁶ ST expression of angiogenic inductors,⁷ the central role of the proinflammatory cytokine tumour necrosis factor α in its pathogenesis,⁸ and RANKL derived mechanisms of bone erosion.⁹ Although p53 overexpression has been recorded in the cutaneous lesions of psoriasis,¹⁰ studies focusing on p53 expression in the ST of patients with PsA are lacking.

This study aimed at analysing p53 protein expression and different cell markers in ST from patients with RA and PsA and its association with radiographic damage.

PATIENTS AND METHODS

Patients

Forty five consecutive patients (24F/21M) with active arthritis attending the outpatient clinic of the rheumatology

department of the Hospital Clínic of Barcelona were included. Twenty seven fulfilled the American College of Rheumatology criteria for RA and 18 fulfilled the Moll and Wright criteria for PsA. Laboratory tests included serum C reactive protein levels (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF; by nephelometry), and HLA-B27 status (by polymerase chain reaction). All patients underwent diagnostic or therapeutic arthroscopy of one affected joint after giving informed consent. Radiographs of hands, feet, and the joint undergoing arthroscopy were obtained in all cases at inclusion in the study. Hands and feet radiographs were obtained after 12 months of follow up in a subgroup of patients with RA with less than 12 months of disease duration. The study was approved by the ethics committee of the Hospital Clínic.

Arthroscopy and synovial biopsies

Arthroscopies were performed in a sterile room, with local anaesthesia and without knowledge of the radiographic status of the joint undergoing arthroscopy. We used a 2.7 mm arthroscope for knees, wrists, and elbows and one of 1.9 mm for metacarpophalangeal joints (Storz, Tuttlingen, Germany).¹¹ Between six and eight biopsy samples were taken from the affected synovium. Synovial samples were fixed in 10% neutral buffered formalin, dehydrated, and then embedded in paraffin using an automated tissue processor.

Immunohistological analysis

p53

The immunohistological method used to determine p53 expression has been previously described.³ Briefly, paraffin

Abbreviations: CRP, C reactive protein; ESR, erythrocyte sedimentation rate; mAb, monoclonal antibody; PsA, psoriatic arthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; ST, synovial tissue

Table 1 Clinical and serological characteristics of patients with RA and PsA

	Rheumatoid arthritis (n = 27)	Psoriatic arthritis (n = 18)
Women, No (%)	16 (59)	8 (44)
Age (years), mean (SD)	54.1 (15.6)	44.9 (13.1)
Disease duration (months), mean (SD)	60.3 (90)	88.6 (82.1)
RF+, No (%)	19 (70)	0 (0)
HLA-B27 +, No (%)	2 (7)	1 (6)
Baseline erosions*, No (%)	12 (44)	6 (33)
ESR (mm/1st h), mean (SD)	35.8 (31.2)	43.7 (27.5)
CRP (mg/l), mean (SD)	23 (35)	26 (29)

*Number of patients with radiological erosions at the time of arthroscopy.

sections of 4–6 µm were immunostained with mouse anti-p53 monoclonal antibody (mAb) DO7 (Novocastra Laboratories, Newcastle, UK) diluted to a final concentration of 0.2 µg/ml. As secondary antibodies, donkey antimouse antibodies labelled with biotin (Jackson Immuno-Research, West Grove, PA) were diluted to a final concentration of 5 µg/ml and incubated for 30 minutes. Incubation with SA-horseradish peroxidase for 30 minutes was followed by incubation with biotinylated tyramide (TSA Biotin System, Perkin-Elmer, Boston, MA).

As previous studies carried out this amplification method using frozen preparations, a confirmatory test for optimal p53 detection in paraffin sections using cyanine 3-fluorophore labelled tyramides (TSA Fluorescence System, Perkin-Elmer), a method in which we have experience, was performed as previously.¹² Colon adenocarcinoma tissue preparations served as a positive p53 control. In each procedure, additional sections were incubated with only the secondary antibody and detection system to exclude non-specific binding.

Cellular infiltrate

Staining of serial sections was performed using the following mAbs: anti-CD4 (1F6; Novocastra), anti-CD8 (4b11; Novocastra), anti-C20 (L26; Dako), anti-CD68 (KP-1; Dako), and anti-CD138 (B-B4; Santacruz, San Diego, CA), as previously described.⁷

Microscopic analysis

The p53 protein was detected and scored in the lining, sublining, and endothelium of ST. A p53 total score was also obtained. All sections were randomly evaluated by two independent observers who were unaware of the clinical

data. The compensated κ value of interobserver reliability was 0.85. Immunohistochemical results for p53 expression were scored as previously reported.³

Expression of the different cell markers (CD4, CD8, CD20, CD68, and CD138) was scored semiquantitatively.⁷ Scores were individualised for each marker.

Radiological analysis

At study entry, radiographs of hands, feet, and the joint undergoing arthroscopy were obtained in all cases, in order to classify patients' disease as erosive or non-erosive. Furthermore, hands and feet radiographs were made after 1 year of follow up in 11/18 patients with RA. This subgroup of patients was included because their disease duration was <12 months (mean (SD) 6.4 (3.5) months). The remaining seven patients had a disease duration >36 months (mean (SD) 146 (97.6) months). A change of two or more units using the Larsen-Scott method was used to define 1 year radiographic progression in these patients.^{13 14} All radiographs were scored by the same observer chronologically.

Statistical analysis

Data were analysed using the SPSS 10.0 statistical software package, under licence to the Hospital Clinic of Barcelona. The univariate analysis was performed using the χ^2 test, Student *t* test, or the non-parametric Mann-Whitney test. The non-parametric Spearman correlation between biological parameters (CRP and ESR) and the p53 protein was calculated.

Risk association, adjusted for CRP and ESR, between the p53 protein and the presence of RA was estimated by multivariate logistic regression. The level of statistical significance was established at $p \leq 0.05$.

RESULTS

Clinical features

Forty five patients: 27 RA (16F/11M, 19 (70%) RF+, mean (SD) age 54.1 (15.6) years, mean disease duration 60.3 (90) months) and 18 PsA (8F/10M, mean (SD) age 44.9 (13.1) years, mean disease duration 88.6 (82.1) months), 11 with RF negative polyarthritis and seven with oligoarthritis, were included in the study. There were no significant differences in disease duration between the two groups of patients. Arthroscopy was performed in the knee (n = 35), wrist (n = 8), elbow (n = 1), and metacarpophalangeal joints (n = 1). At inclusion, most patients were treated with non-steroidal anti-inflammatory drugs and low doses of prednisone (mean daily dose 5 mg). Fifteen patients in the RA group also received disease modifying antirheumatic drugs

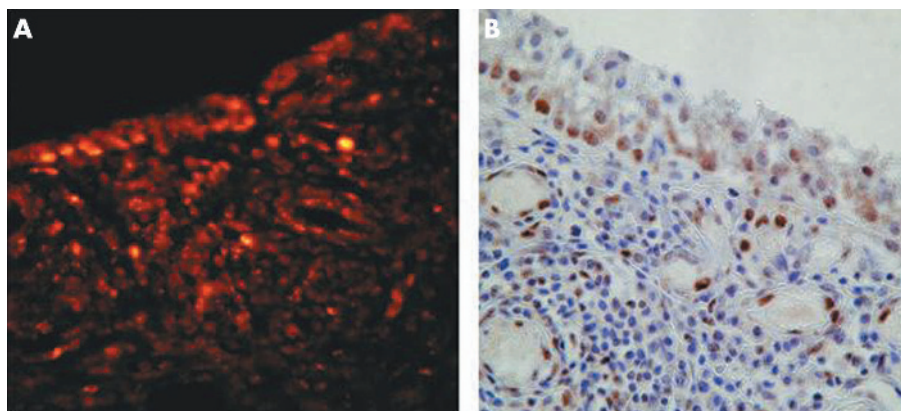


Figure 1 Immunofluorescence (A) and immunohistochemical (B) staining for p53 in ST of the same patient with RA. Technique with tyramide enhancement (see text). Original magnification $\times 600$ (A and B).

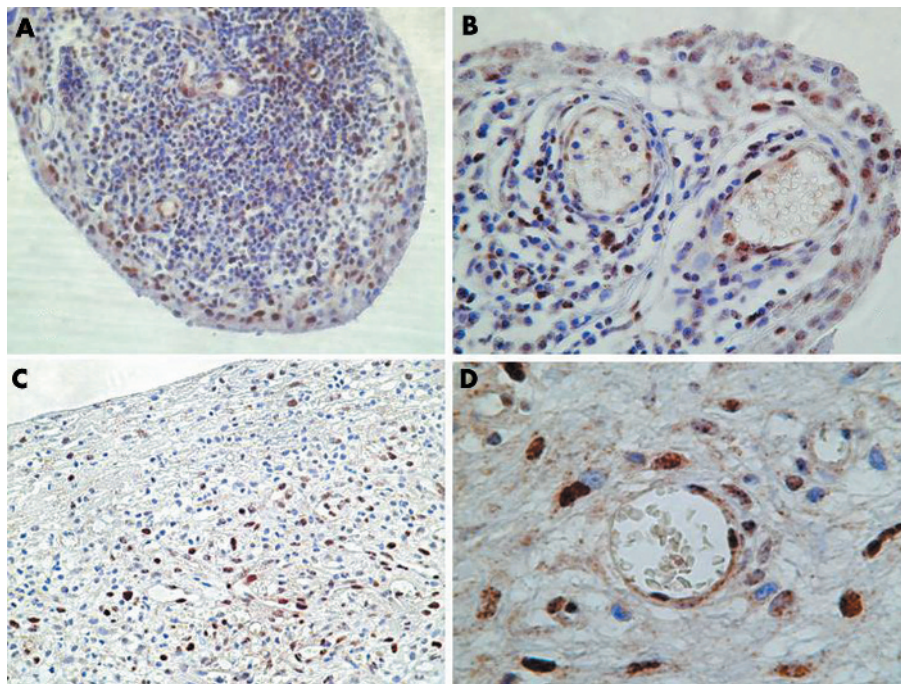


Figure 2 Immunostaining for p53 tissue in ST from patients with RA (A and B) and PsA (C and D). Original magnification $\times 400$ (A); $\times 600$ (B); $\times 400$ (C), and $\times 1000$ (D).

(9 (60%) intramuscular gold salts, and 6 (40%) methotrexate) and 12 patients in the PsA group were treated with methotrexate. Table 1 shows the demographic and clinical characteristics of the patients.

Radiological data

Eighteen (12 RA/6 PsA) of 45 (40%) patients had radiological erosions at the moment of arthroscopy. As expected, the percentage of erosive disease in RA was significantly greater in patients with established disease than in those with RA of <12 months: 87.5% v 26%. No significant differences in biological parameters of inflammation (CRP or ESR) were seen between patients with and without erosions (table 1). Radiographic progression was found in 4/11 patients with RA in whom 1 year radiological progression was analysed. At the end of follow up, all these patients were treated with disease modifying antirheumatic drugs: four with methotrexate, six with parenteral gold, and one with hydroxychloroquine.

Immunohistology of p53

The final p53 protein immunohistochemical analysis included 34 (18 RA, 16 PsA) of 45 patients; 11 patients were excluded because their tissue preparations, principally

Table 2 p53 expression* in different areas of synovial tissue of patients with RA and PsA

	Rheumatoid arthritis (n = 18)	Psoriatic arthritis (n = 16)	p Value
Global p53	2.0 (1.4)	0.8 (0.9)	0.008
Endothelial p53	2.9 (1.3)	1.6 (1.5)	0.020
Lining p53	3.0 (1.0)	0.9 (1.2)	<0.0001
Sublining p53	2.0 (1.3)	0.8 (1.0)	0.012
ESR (mm/1st h)	38.9 (35.2)	43.7 (27.5)	0.27
CRP (mg/l)	26 (40)	26 (28)	0.78

Results are shown as mean (SD).
*p53 was only scored in 34/45 patients.

Table 3 Clinical and radiological characteristics of patients according to p53 scores

	p53 ≥ 2 (n = 13)	p53 ≤ 1 (n = 21)
Sex (F), No (%)	8 (62)	12 (57)
Age, mean (SD)	55.1 (16.4)	47.1 (13.3)
Mean evolution of disease (months), mean (SD)	75.1 (77)	83.8 (99.3)
Baseline erosions, No (%)	8 (62)	5 (24)
Diagnostic	10RA/3PsA	8RA/13PsA

obtained from wrist and metacarpophalangeal joints, did not include sufficient synovial membrane for adequate p53 evaluation. The p53 immunofluorescence staining and peroxidase staining were concordant and positive in 16/18 patients with RA and in 9/16 patients with PsA (fig 1). Staining was nuclear and cytoplasmic (fig 2), but only p53 protein stained with the peroxidase technique was scored.

p53 expression in RA and PsA

The mean (SD) scores for total p53 expression were significantly higher in patients with RA than in patients with PsA (2.0 (1.4) v 0.8 (0.9); p = 0.008). The highest scores, 2, 3, and 4 (>5% of positive cells), were found in 10/18 (56%) patients with RA compared with 3/16 (19%) patients with PsA. A score of 4 was found in 4/18 (22%) patients with RA, but in no patient with PsA. Conversely, a p53 total score of 0 was found in 2/18 (11%) patients with RA compared with 7/16 (44%) patients with PsA.

The scores for p53 expression in the lining, sublining, and endothelium were all also significantly higher in patients with RA than in patients with PsA (table 2).

The presence of p53 expression scores ≥ 2 in all the areas analysed, adjusted by inflammation parameters (CRP and ESR), conferred an increased risk of having RA, with an odds ratio = 8.2 (95% confidence interval 1.4 to 49.3; p = 0.022) for

Table 4 Expression of different cell markers in synovial tissue of patients with RA and PsA.

	Rheumatoid arthritis (n = 27)	Psoriatic arthritis (n = 18)	p Value
CD4	2.9 (0.7)	2.1 (1.0)	0.012
CD8	2.5 (1.0)	2.2 (0.9)	0.459
CD20	2.0 (1.4)	1.5 (1.3)	0.235
CD138	1.5 (1.4)	1.4 (1.1)	0.971
CD68	2.0 (1.3)	1.7 (0.96)	0.117

Results are shown as mean (SD).

p53 total expression. The highest risk was with lining p53 expression (odds ratio = 31.5 (95% confidence interval 4.5 to 220.2; $p = 0.001$).

p53 and radiographic damage

Total and sublining p53 expression was associated with erosive disease ($p = 0.012$ and $p = 0.032$, respectively). However, this association was only present in the RA group: the scores of the p53 total, lining, sublining, and endothelial expression were significantly higher in patients with erosive RA than in those with non-erosive disease. Furthermore, none of the patients with erosive RA had a score of 0, and 60–100% of patients with erosive RA had scores of 3 or 4 in the synovial areas analysed. Table 3 shows the clinical and radiological characteristics of the 34 patients according to p53 scores.

When analysing the 11 patients with RA in whom 1 year radiographic follow up was available, p53 synovial expression score was 4 in 3/4 (75%) patients with progression, whereas 6/7 (86%) patients without progression scored ≤ 1 . In all three patients with significant progression after 1 year (Larsen >10 units), p53 expression score was the maximum (4).

No association was found between p53 expression and erosive PsA, and the distribution of score 0 or scores 3–4 was similar in patients with erosive and non-erosive PsA.

Immunohistology of cellular markers

Only the mean score for T CD4+ lymphocytes was significantly higher in patients with RA than in those with PsA ($p = 0.012$) (table 4). The mean (SD) CD68+ cell expression score was significantly higher in patients with baseline erosions than in those without erosions (2.6 (1.1) v 1.8 (1.1); $p = 0.03$). However, this association was only valid for the RA group: erosive RA had significantly higher CD68+ scores than patients with non-erosive RA (3.1 (1) v 1.9 (1.3); $p = 0.03$). Furthermore, 73% of patients with erosive RA had CD68+ scores of 3–4 compared with only 23% of patients with non-erosive RA. CD68+ expression mean score in the four patients with 1 year radiological progression was 3, whereas in the seven patients without radiological progression the score was 1.7.

DISCUSSION

In this study p53 was detected in almost all patients with RA in the synovial lining, sublining, and endothelium. We found an association between the mean p53 score and erosive disease at baseline in patients with RA. In addition, around 80% of patients with RA with erosion had the maximum p53 scores. Radiological progression after 1 year in RA was also associated with the highest p53 scores at study entry. These results confirm previous studies on p53 expression in the ST of patients with RA,^{1 3} and show, for the first time, a consistent association between p53 expression and joint damage. Other studies have not found significant p53

expression in RA ST, probably because less sensitive methods were used.^{15 16}

On the other hand, this study found that p53 is also expressed in PsA ST, although at a significantly lower level than in RA, and that this expression is not associated with joint damage. Only one previous study, published in abstract form, reported p53 expression in the nuclei of lining cells of six patients with PsA, although it provided no methodological, clinical, or p53 scoring data to compare with our study.¹⁷

The differential expression of p53 in RA and PsA in this study did not seem to be related to differences in disease duration or inflammatory activity, two factors that might affect p53 expression.⁴ This difference was maintained when each area of ST scored for p53 expression was compared between RA and PsA.

Our results suggest that p53 expression has distinct pathogenic consequences in RA compared with PsA because an association with joint damage was found only in RA, both at baseline and after 1 year of follow up. However, given the small number of patients prospectively analysed, this point needs to be confirmed in future studies. Another limitation of this study is that our results are principally based on knee synovium, because samples obtained from the wrist and metacarpophalangeal joint were insufficient to obtain a complete p53 score. Similar studies based on biopsy specimens obtained from small joints might be interesting in order to extend our results.

Although there are studies on p53 mutations and protein expression in RA and other inflammatory disorders,^{1–3 18–20} and much fine basic research has been done on the topic,^{4 21–23} the clinical significance of p53 expression in RA remains unclear. Originally, overexpression of p53 in tissues was thought to be a surrogate marker for mutation. The demonstration of p53 single nucleotide mutations in the synovium of patients with RA, predominantly in the lining layer, suggested that the oxidative environment of rheumatoid synovium induced these mutations.^{2 18} Subsequently, the same authors also reported high p53 expression in the sublining.³ Muted p53 proteins have a longer half life than wild ones, which increases their expression. Furthermore, muted p53 might lose its growth control regulatory properties and might even induce synthesis of metalloproteinases and proinflammatory cytokines, causing joint damage.²⁴ However, recent studies have found wild-type p53 in human inflammatory diseases, including RA, reactive arthritis, and psoriasis,²⁰ and this has been confirmed in animal models of arthritis.⁴ One explanation for these findings would be that total p53 expression in RA includes muted and wild forms, with muted p53 predominating in the lining and leading to a net destructive effect. On the other hand, p53 expression in other inflammatory rheumatic diseases, including PsA, might be wild-type, with a protective role. However, this important question should be examined by specific p53 mutation studies.

A recent study using synovial RA fibroblasts and the non-inflammatory SCID mouse co-implantation model of RA, suggests that p53 at sites of cartilage invasion could be induced during the destructive process driven by activated RA synovial fibroblasts, without the participation of inflammatory cells.²³ Although obtained in an animal model, these results agree with the finding that p53 is overexpressed in the lining of RA, the site of previously demonstrated mutations, and that its expression correlates with joint damage.

Although PsA may share several pathogenic mechanisms with RA,^{6–9} our study suggests that differential pathogenic mechanisms of joint damage exist between RA and PsA. In PsA, erosions occur less commonly than in RA and progression to joint destruction occurs at a slower rate.²⁵ Some authors have suggested that synovitis in PsA might be a

secondary phenomenon to enthesitis or osteitis,²⁶ or that synovitis may not be responsible for the destructive lesions characteristic of PsA.²⁷ In addition, it has also been reported that angiogenesis plays a fundamental part in the pathophysiology of synovitis in PsA as compared with RA,²⁸ and that the vascular synovial morphology is distinct in PsA and RA.^{11, 29} However, in a recent study, the different degrees of radiological progression of PsA and RA were not explained by a difference in the expression of matrix metalloproteinases in synovium, suggesting that the development of bone and cartilage damage is a complex process influenced by multiple factors.³⁰

Finally, this study confirms previous reports on the association of the degree of synovial macrophage infiltration with joint damage in RA.³¹ Accumulation of macrophages in the lining with consequent production of cytokines and matrix metalloproteinases has been implicated in erosive joint damage in RA.³² These results, together with the p53 data, lend additional support to the hypothesis that there are differential pathogenic consequences of synovitis in PsA and RA.

In conclusion this study is, to our knowledge, the first to confirm differential p53 expression in the ST of patients with RA and PsA, as well as an association of p53 expression with joint damage in RA. At the same time, our study suggests differential pathogenic consequences of synovitis in RA and PsA and points to p53 expression as a prognostic marker of joint damage in RA, underlining the need for future confirmative studies.

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