

# Quantitation of Metalloproteinase Gene Expression in Rheumatoid and Psoriatic Arthritis Synovial Tissue Distal and Proximal to the Cartilage-Pannus Junction

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**ABSTRACT. Objective.** The distinct and different patterns of radiological damage in psoriatic arthritis (PsA) and rheumatoid arthritis (RA) may be a product of the relative balance of proteolytic enzyme and inhibitor gene expression in synovial tissue. This study compared metalloproteinase gene expression in synovium located proximal to the cartilage-pannus junction (CPJ) and distal to the CPJ (non-CPJ) in patients with PsA and RA.

**Methods.** Synovial biopsies were obtained from CPJ and non-CPJ sites under direct vision at arthroscopy of an inflamed knee in patients with PsA (n = 12) and RA (n = 12) who were not under disease modifying antirheumatic drug treatment. A competitive, quantitative RT-PCR technique was established for synovial RNA using a polycompetitor construct containing mRNA-specific primer sites for collagenase (MMP-1), stromelysin (MMP-3), tissue inhibitor of metalloproteinase-1 (TIMP-1), and GAPDH. cDNA products were separated and quantified by ethidium bromide stained gel electrophoresis and mRNA values were normalized relative to GAPDH expression.

**Results.** MMP-1, MMP-3, and TIMP-1 mRNA were upregulated in RA and PsA synovium with a prodestructive (MMP-1 + MMP-3)/TIMP-1 balance in both diseases. Similar levels of MMP mRNA expression were observed in PsA and RA despite the presence of less radiological erosion in the PsA group. No difference was observed in the degree of upregulation of MMP-1, MMP-3, or TIMP-1 mRNA in paired biopsies from CPJ and non-CPJ sites in either PsA (n = 8) or RA (n = 10). The ratio of TIMP-1 expression in CPJ compared to non-CPJ biopsies was higher in patients with nonerosive disease ( $10.1 \pm 27.8$ ) than in erosive patients ( $0.75 \pm 0.27$ ;  $p = 0.07$ ).

**Conclusion.** PsA and RA have similar levels of MMP-1, MMP-3, and TIMP-1 mRNA expression in synovium. There is no evidence of increased metalloproteinase mRNA expression at the CPJ in RA or PsA. The different patterns of radiological progression seen in RA and PsA were not explained by differences in synovial mRNA expression of MMP-1, MMP-3, or TIMP-1. (J Rheumatol 2004; 31:1274–80)

## Key Indexing Terms:

METALLOPROTEINASE  
PSORIATIC ARTHRITIS

RHEUMATOID ARTHRITIS  
CARTILAGE-PANNUS JUNCTION

Psoriatic arthritis (PsA) and rheumatoid arthritis (RA) are characterized by synovial inflammation that may subsequently lead to cartilage and bone destruction<sup>1</sup>. There are interesting differences in the radiological features of PsA and RA that make them ideal comparators for study of inflammation and cartilage destruction in the synovial joint.

In PsA, erosions occur less commonly than in RA, progression to joint destruction occurs at a slower rate<sup>2</sup>, and the distinguishing radiological features are periostitis and resorption, which occur more distally to the articular surfaces, possibly at the sites of enthesal insertion<sup>3</sup>. The pattern of radiological damage in RA is characterized by cartilage and bone erosion at the cartilage-pannus junction (CPJ)<sup>4</sup>. These differences may be explained by a quantitative or qualitative difference in the production of mediators of joint destruction or their inhibitors, in particular at the site of maximal joint destruction — the CPJ<sup>5-7</sup>.

Matrix metalloproteinases (MMP) are a family of proteolytic enzymes that are capable of degrading all components of the extracellular matrix, a key event in the development of cartilage destruction and joint erosion<sup>8</sup>. At least 4 groups of MMP exist: (1) collagenases, (2) gelatinases, (3) stromelysins, and (4) membrane associated MMP. Tissue inhibitors of the MMP (TIMP) are also produced within the joint and it is the balance of production of MMP and TIMP

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that is believed to determine the development of joint destruction in inflammatory arthritis<sup>8,9</sup>. Further, therapies that are effective at retarding radiological progression in RA have been shown to result in a reduction in MMP expression in synovial tissue<sup>10-12</sup>. This suggests that MMP play a key role in the development of erosion at the CPJ.

Collagenase (MMP-1) has a key role in the pathogenesis of joint destruction in RA as collagenase activity is the rate-limiting step in the breakdown of collagen<sup>13</sup>, a principal constituent of articular hyaline cartilage. Concentrations of MMP-1 are increased in serum<sup>14,15</sup>, synovial fluid<sup>16</sup>, and synovial membrane<sup>17,18</sup> in RA. It is present at sites of bone and cartilage erosion<sup>19,20</sup>, and expression of MMP-1 in serum and MMP-1 mRNA in synovium in early RA correlates with the development of radiological erosions<sup>15,21</sup>. Fewer data exist on MMP-1 expression in PsA. Similar serum levels of MMP-1 are reported in established PsA and RA<sup>14</sup>. In early PsA, MMP-1 mRNA is upregulated in non-CPJ synovium, with levels of expression similar to RA<sup>22</sup>, although collagenolytic activity of PsA synovial fluid may be greater than that of RA<sup>23,24</sup>. Stromelysin (MMP-3) and TIMP-1 are also elevated in serum<sup>14</sup>, synovial fluid<sup>16,25</sup>, and at CPJ and non-CPJ synovial membrane in RA<sup>17,18,20</sup>. Serum concentrations of MMP-3 correlate with measures of systemic inflammation in RA<sup>14,26</sup>, although the expression of MMP-3 and TIMP-1 in the serum did not correlate with the development of erosions of hands and feet<sup>15</sup>. Serum levels of MMP-3 and TIMP-1 are similar in PsA and RA<sup>14</sup>. No data exist for MMP-3 or TIMP-1 mRNA expression in synovium in PsA.

While MMP-1, MMP-3, and TIMP-1 have been implicated in the pathogenesis of both RA and PsA<sup>14</sup>, no previous study has quantified and compared their mRNA expression in synovium. In addition, no study has compared their mRNA expression in synovium obtained from sites distal and proximal to the CPJ. Similar levels of MMP-1 and MMP-3 have been detected immunohistochemically in suprapatellar and CPJ synovium in RA<sup>20</sup>. This technique does not differentiate active and latent MMP, whereas the presence of MMP mRNA indicates active gene transcription. In order to measure MMP-1, MMP-3, and TIMP-1 mRNA expression in synovium, a polycompetitor quantitative reverse transcription polymerase chain reaction (RT-PCR) technique was established and validated. This technique was then used to quantify MMP gene expression in synovial samples obtained arthroscopically from sites distal and proximal to the CPJ in patients with PsA and RA.

## MATERIALS AND METHODS

*Clinical and radiological assessment of patients.* PsA was diagnosed using the criteria of Veale, *et al*<sup>27</sup>; RA was diagnosed using the American College of Rheumatology criteria<sup>28</sup>. All patients were required to have active knee synovitis for entry to the study. A 44 swollen joint count and Ritchie index<sup>29</sup> was performed by the same physician (DK) on the day of arthroscopy. Erythrocyte sedimentation rate (ESR) was measured using the standard

Westergren technique; serum C-reactive protein (CRP) was measured by standard nephelometry.

Posteroanterior plain radiographs of the hands, wrists, and feet were only obtained in patients in whom paired CPJ and non-CPJ biopsies were obtained and were performed within 4 weeks of the synovial biopsy. Radiographs were evaluated by a single observer (DK) blinded to patient identity of radiographs using the Sharp/Van der Heijde method<sup>30,31</sup>. Using this score the maximum number of erosions in the hands is 160 and the maximum number of erosions in the feet is 120; the maximum scores for joint space narrowing are 120 and 48, respectively.

*Arthroscopic biopsy and RNA extraction.* Ethical approval was obtained from the St. Vincent's University Hospital ethics committee. Synovial biopsy was performed using a 2.7 mm Storz arthroscope and a grasping forceps<sup>19,20</sup> under direct vision at arthroscopy of actively inflamed knee in patients with PsA and RA who were not taking disease modifying antirheumatic drugs (DMARD). All compartments of the knee were visualized. Biopsies were obtained from sites distal to the CPJ (suprapatellar, infrapatellar, medial and lateral compartments) and from synovium immediately proximal to the CPJ at the femoral trochlea (defined as a biopsy taken as close to CPJ as possible without biopsying the cartilage). Synovial biopsies were immediately snap-frozen and stored at  $-70^{\circ}\text{C}$  until used. Biopsies obtained from the same sites were also processed for histology to confirm that synovial tissue was obtained in all cases. Total RNA was extracted from synovial tissue after mechanical homogenization by the guanidium isothiocyanate method and resuspended in diethyl pyrocarbonate treated water. The concentration of total RNA was determined by UV absorption at 260 nm. The quality and purity of total RNA was confirmed on 2% agarose gel electrophoresis and by UV absorption at 260 and 280 nm.

*Generation of polycompetitor construct.* A RT-PCR polycompetitor for use in quantifying acute phase serum amyloid A, constitutive serum amyloid A, serum amyloid P component, CRP, apolipoprotein A1, apolipoprotein A2, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and  $\beta$ -actin mRNA was previously generated and validated in quantitative PCR<sup>32</sup>. This polycompetitor was modified to incorporate additional primer sequences for MMP-1, MMP-3, TIMP-1, interleukin 1 $\beta$  (IL-1 $\beta$ ), IL-1 receptor antagonist (IL-1ra), IL-6, IL-10, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). This technique allows multiple genes to be accurately quantified in small tissue samples without the need for specialized real-time PCR equipment.

Gene sequences were retrieved from Genbank. The following accession numbers were used: MMP-1 (J05070), MMP-3 (X05232), TIMP-1 (X03124), IL-1 $\beta$  (K02770), IL-1ra (X52015), IL-6 (s56892), IL-10 (M57627), TNF- $\alpha$  (M10988). Primer sets (sequences available on request) were designed using the Primer program (v. 0.5, The Whitehead Institute for Biomedical Research, Cambridge, MA, USA). Primers were designed towards sequence in separate exons so that cDNA products generated from DNA could be distinguished from cDNA products generated from DNA based on size difference. Each primer pair was designed to generate a cDNA product of approximately 400 base pairs (bp) and to have a  $T_m$  of  $60^{\circ}\text{C}$  with a GC content of 40–60%. PCR conditions were optimized for each primer pair with different concentrations of magnesium chloride and different annealing temperatures.

The original polycompetitor construct<sup>32</sup> was designed such that products generated by PCR using "mRNA-specific" primer pairs are roughly 100 bp smaller than those derived from the endogenous cellular/tissue mRNA. The size difference allows for separation of polycompetitor and tissue cDNA products, but did not exceed 100 bp to ensure equal rates of amplification of competitor and tissue mRNA during PCR. Primer sites specific for the new target genes were introduced into the existing polycompetitor using the multiple cloning sites therein<sup>32</sup> (Figure 1). After design of the modified polycompetitor construct, all the selected primer sequences and the modified polycompetitor construct were searched with the Wisconsin GCG package version 8.1-UNIX for nonspecific priming sites that could lead to PCR artefacts. The modified construct, polycompetitor-2 (PC-2) was then fully sequenced to confirm the sequence (available on request).

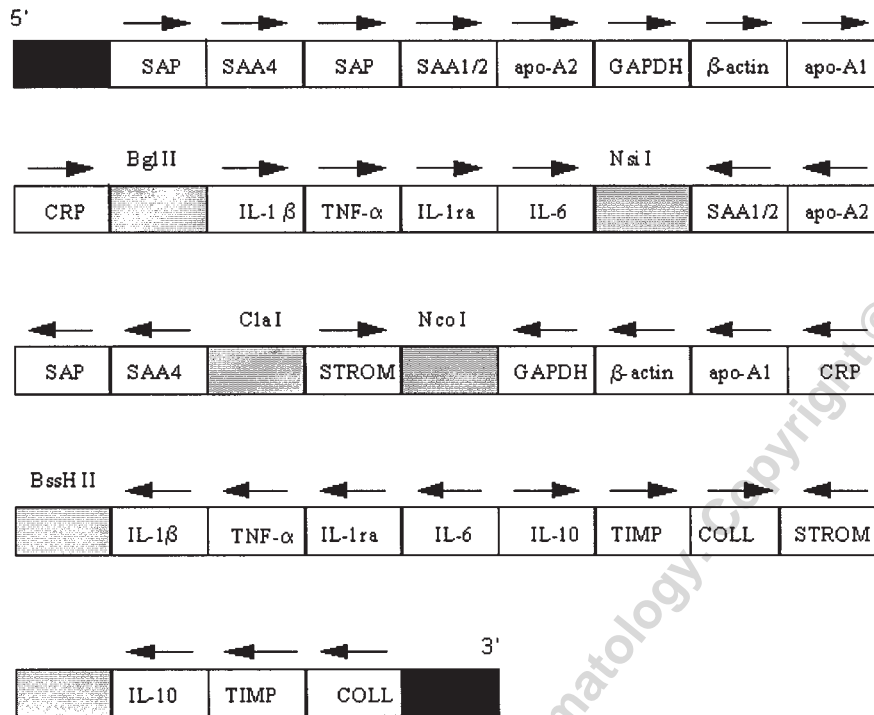


Figure 1. The sequence of forward and reverse primer sites in the modified polycompetitor construct allows quantitation of multiple genes from RNA derived from synovial samples.

Double stranded PC-2 DNA was linearized with EcoRI, gel purified, and ethanol precipitated, then roughly 7 µg of DNA in a 100 ml reaction was used for *in vitro* transcription with SP6 polymerase (Promega, Madison, WI, USA) to generate polycompetitor RNA. PC-2 RNA was treated with DNase, purified by phenol/chloroform extraction, recovered by ethanol precipitation, resuspended in 300 µl diethyl pyrocarbonate treated water, aliquoted, and stored at -70°C until use. The quality of the expressed RNA was checked on a denaturing agarose/formaldehyde gel.

**RT-PCR.** RT-PCR was performed according to a "continuous RT-PCR" protocol adapted from Mallet, *et al*<sup>33</sup>. RT-PCR was in a buffer initially containing 10 mM Tris-HCl, 50 mM potassium chloride, 0.1% (v/v) Triton X100, 0.2 mM of each dNTP, 7.5–12.5 mM MgCl<sub>2</sub>, 100 ng of each primer, 0–15% (v/v) dimethyl sulfoxide, 1 U/ml Anti-RNase (Ambion, Austin, TX, USA), 0.2 U Taq-polymerase, and 0.2 U AMV-reverse transcriptase (Roche, Indianapolis, IN, USA), pH 8.5, in a 25 µl reaction mixture. The one-step RT-PCR was performed with a spectrophotometrically quantified dilution series of polycompetitor RNA (4 µg/ml at dilutions of 1/25, 1/50, 1/100, 1/200, 1/500, 1/1500, 1/5000, 1/10,000) in competition with unquantified but equal amounts of total synovial RNA (50 ng) in each reaction. Thermocycling was performed as follows: 30 min at 48°C (annealing and reverse transcription); 5 min at 95°C (inactivation of reverse transcriptase); 1 min at 94°C (denaturation); 1 min at 55–63°C (annealing); 1 min at 72°C (extension); 5 min at 72°C (final extension). Steps 3–5 were performed through 40 cycles.

**Quantitation of RT-PCR products.** RT-PCR products were separated by electrophoresis on a 2% agarose gel (Figure 2), identified by ethidium bromide staining, and quantified by UV absorbance at 254 nm with UVP-GrabIt™ and Gelworld™ software. A log-log plot of the ratios of band densities of competitor and synovial products with a known series of concentrations of competitor RNA was performed (Figure 2). Analysis of this determined the equivalence concentration of synovial mRNA and competitor RNA. Reproducibility is determined to be very high when starting ratios of internal standard and mRNA are at an approximate equiv-

alence point. Relative standard deviations were less than 10% between independent RT-PCR reactions with the same sample mix of internal standard and total RNA. MMP-1, MMP-3, and TIMP-1 mRNA expression was expressed in units as a ratio of GAPDH mRNA in the same sample.

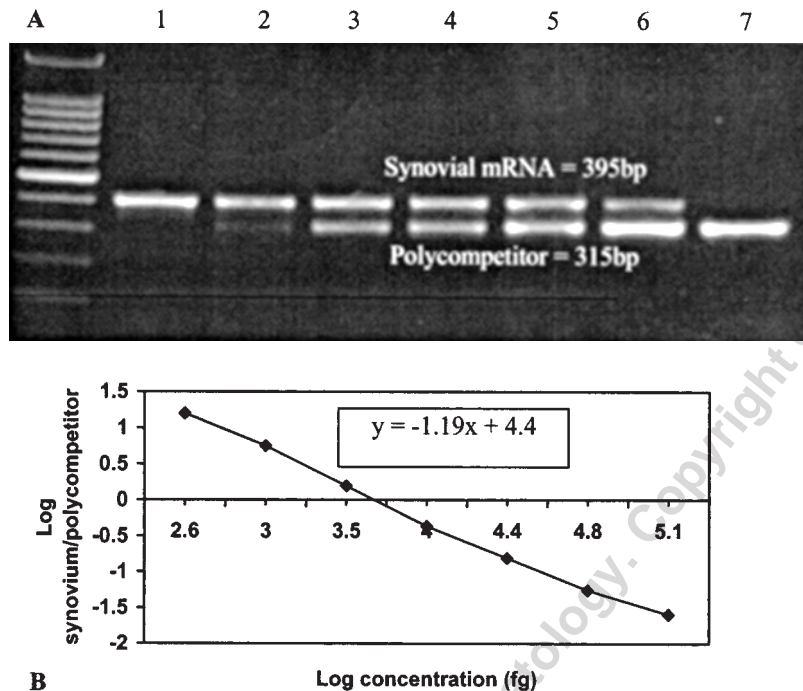
**Statistical analysis.** Statistical analysis was performed using Microsoft Excel 5.0 and Statview™ software. Nonparametric analysis was performed for comparison of medians using the Mann-Whitney U-test. Correlations were determined using the Spearman correlation coefficient.

## RESULTS

**Clinical and laboratory measures (Table 1).** All patients had active knee synovitis and were taking no DMARD at the time of biopsy. The duration of disease was similar in the 2 study groups, with most patients at a relatively early stage of disease. The RA group had a significantly greater number of inflamed joints.

**Radiological assessment.** Radiographs were available in 17 of the 18 patients (PsA = 7, RA = 10) in whom paired CPJ and non-CPJ synovial biopsies were obtained; one set of radiographs was performed but could not be located at the time of evaluation. None of the 7 patients with PsA had erosions of the hands or feet or joint space narrowing, periostitis, or bony resorption. Four of the 10 patients with RA had erosions at the time of synovial biopsy and 2 had joint space narrowing (median Sharp score 23.5, range 2–41).

**MMP-1, MMP-3 and TIMP-1 mRNA in PsA and RA non-CPJ synovium (Table 2).** MMP-1, MMP-3, and TIMP-1 mRNA expression in synovial membrane from non-CPJ



**Figure 2.** A. Quantification of MMP-3 mRNA expression in synovial tissue in a patient with PsA. Products of RT-PCR are separated by size difference on gel electrophoresis. An equal amount of synovial RNA and a variable, but quantified, dilution of PC-2 RNA (4 ng/ml) were amplified by RT-PCR. The dilution series of PC-2 ranges from 1/5000 dilution in lane 1 to 1/25 dilution in lane 7 (1/5000, 1/1500, 1/500, 1/200, 1/100, 1/50, 1/25). A size marker was run on the left side to identify PCR products by their respective sizes (synovial product = 395 base pairs, polycompetitor product = 315 base pairs). B. Quantification plot of band densities obtained in A: The log of the ratio of the measured band densities from the gel are plotted against the log of the concentration of the polycompetitor. The equation of the line allows calculation of the corresponding concentration of polycompetitor RNA when  $y = 0$  (i.e., equivalence of synovial and polycompetitor RNA). Thus for  $y = -1.19x + 4.4$ , when  $y = 0$ ,  $x = 3.67$  and reverse log (3.67) = 4704 fg RNA.

**Table 1.** Clinical details of all patients who underwent synovial biopsy. Data are mean  $\pm$  SEM.

	Psoriatic Arthritis, n = 12	Rheumatoid Arthritis, n = 12	p*
Duration, mo	29.8 $\pm$ 11.1	17.5 $\pm$ 6.7	0.45
RI	5.0 $\pm$ 1.4	14 $\pm$ 2.3	0.003
SJC	5.1 $\pm$ 1.4	15.5 $\pm$ 2.6	0.002
ESR mm/h	28.2 $\pm$ 11.6	47.3 $\pm$ 7.9	0.05
CRP	40.8 $\pm$ 18.4	60.6 $\pm$ 17.6	0.19

RI: Ritchie index. SJC: swollen joint count. \* Mann-Whitney U-test.

regions in the knee joint was present in all subjects in both PsA (n = 12) and RA (n = 12) study groups, regardless of disease duration. Levels of MMP-1, MMP-3, and TIMP-1 mRNA in PsA synovium were not significantly different compared to RA. The ratios of metalloproteinase (MMP-1, MMP-3) to metalloproteinase inhibitor (TIMP-1) mRNA were calculated in order to determine the balance of gene expression in favor of matrix degradation. The ratio of (MMP-1 + MMP-3)/TIMP-1 was greater than 1 in both

disease groups, reflecting a proinflammatory and prode-structive balance of gene expression. TIMP-1 mRNA levels, but not MMP-1 or MMP-3, correlated positively with ESR ( $r = 0.57$ ,  $p = 0.004$ ) and CRP ( $r = 0.46$ ,  $p = 0.03$ ) in both RA and PsA.

*MMP-1, MMP-3, and TIMP-1 mRNA in paired CPJ and non-CPJ synovium.* Paired synovial biopsies from CPJ and non-CPJ synovium were obtained in 8 patients with PsA (Table 3) and 10 patients with RA (Table 4). CPJ tissue was not obtained in 6 patients, because of inadequate visualization of the CPJ at arthroscopy or patient intolerance of CPJ biopsy or insufficient tissue obtained at biopsy. MMP-1, MMP-3, and TIMP-1 mRNA expression was detected at both CPJ and non-CPJ sites in both disease groups. There was no significant difference between CPJ and non-CPJ expression of MMP-1, MMP-3, or TIMP-1 or ratios of MMP-1/TIMP-1, MMP-3/TIMP-1, or (MMP-1 + MMP-3)/TIMP-1 in PsA or RA groups analyzed separately or together (data not shown).

*Correlation of radiological and clinical measure and MMP mRNA expression.* Patients with erosions of hands and feet



Table 2. Metalloproteinase mRNA expression in non-CPJ synovium in PsA and RA. All mRNA expressed relative to GAPDH, values as median (range).

	Psoriatic Arthritis, n = 12	Rheumatoid Arthritis, n = 12	p*
Collagenase (MMP-1)	5.4 (1.7–179)	3.5 (0.5–9.1)	0.73
Stromelysin (MMP-3)	9.2 (1.7–207)	14 (0.6–43.2)	0.12
TIMP-1	5.1 (0.4–209)	7.5 (1.1–32)	0.39
MMP-1/TIMP-1	0.9 (0.2–215)	0.3 (0.05–1.4)	0.86
MMP-3/TIMP-1	1.7 (0.05–12)	1.8 (0.04–6.1)	0.07
MMP-1 + MMP-3/TIMP-1	2.9 (0.3–227)	1.9 (0.2–7)	0.34

\* Mann-Whitney U-test.

Table 3. Metalloproteinase mRNA expression in paired CPJ and non-CPJ synovium in PsA (n = 8). All mRNA expressed relative to GAPDH, values as median (range).

	Non-CPJ	CPJ	p*
Stromelysin	8.3 (3–207)	90 (4–269)	0.17
Collagenase	2.7 (1.7–179)	7.4 (0.4–66)	0.59
TIMP	4.5 (0.4–30)	23 (2.3–70)	0.84

\* Mann-Whitney U-test.

Table 4. Metalloproteinase mRNA expression in CPJ and non-CPJ synovium in RA (n = 10). All mRNA expressed relative to GAPDH, values as median (range).

	Non-CPJ	pCPJ	p*
Stromelysin	12.6 (0.6–43)	10.6 (1.6–51)	0.54
Collagenase	2.7 (0.5–9.1)	1.3 (0.1–20)	0.29
TIMP	7.9 (1.1–32)	7.5 (1.2–11.3)	0.41

\* Mann-Whitney U-test.

had a higher swollen joint count ( $20.3 \pm 1.7$  compared to  $8.6 \pm 9.3$ ;  $p = 0.01$ ) and Ritchie Index ( $19.8 \pm 3.9$  compared to  $6.6 \pm 6.7$ ;  $p = 0.02$ ) compared to patients without joint erosions. Similar levels of MMP-1 or MMP-3 mRNA expression were noted in patient groups with and without erosions. The ratio of TIMP-1 in CPJ compared to non-CPJ biopsies was higher in nonerosive patients ( $10.1 \pm 27.8$ ) than in erosive patients ( $0.75 \pm 0.27$ ), but this did not quite reach statistical significance ( $p = 0.07$ ). No difference in clinical or metalloproteinase gene measures was noted between patients with or without joint space narrowing. The same analyses were performed separately for erosions in the hands and in the feet and no significant difference was observed.

## DISCUSSION

The erosion of cartilage and bone in inflammatory arthritis is believed to be mediated largely by collagenase (MMP-1) and stromelysin (MMP-3)<sup>34</sup>. This study quantified MMP

gene expression in synovium in order to determine whether the different rates and patterns of bone and cartilage erosion in PsA and RA may be explained by relative differences in gene expression of metalloproteinases and their inhibitors in the synovial tissue lying immediately proximal (CPJ) and distal (non-CPJ) to the synovial pannus junction with bone and cartilage. Patients with PsA and RA who were not taking DMARD were found to have similar levels of increased MMP-1, MMP-3, and TIMP-1 mRNA expression in CPJ and non-CPJ knee synovium. This confirms the findings of previous studies of plasma<sup>14</sup> and in situ hybridization quantification of collagenase, cathepsin B, and cathepsin L in non-CPJ synovial membrane<sup>22</sup>. This suggests that there is an equal potential for synovial metalloproteinase production in RA and PsA. The ratio of MMP to TIMP-1 mRNA production in both groups was greater than 1, indicating a proinflammatory and prodestructive balance of gene expression in the synovium in PsA and RA. Thus this study provides further evidence that MMP-1 and MMP-3 are likely to be important in the pathogenesis of PsA and thus represent potential therapeutic targets in PsA.

Patients with RA develop more erosive joint damage than patients with PsA<sup>3</sup>, and the accumulation of macrophages with consequent production of cytokines and MMP at the CPJ have been implicated in this<sup>5,6</sup>. High levels of synovial MMP-1 gene expression in non-CPJ synovium in RA — but not in PsA — have been associated with progression of joint erosions<sup>21</sup>. The lack of association in PsA was suggested to relate to the relative balance of metalloproteinases and their inhibitors. Alternatively, the balance of MMP and TIMP-1 expression at the CPJ may be more relevant in the pathogenesis of joint erosion, and non-CPJ expression in RA may have been an indirect measure of this. This is the first study to quantify and compare MMP and TIMP mRNA expression at sites distal and proximal to the CPJ in RA and PsA. Increased MMP-1, MMP-3, and TIMP-1 gene expression was observed in CPJ synovium in both RA and PsA, suggesting that they do play a role in the destruction of bone and cartilage at the CPJ in PsA and RA. However, the different degrees of radiological progression of PsA and RA were not explained by a difference in absolute or relative

levels of MMP or TIMP gene expression in CPJ and non-CPJ synovium, suggesting that other factors also influence the development of bone and cartilage damage.

Posttranscriptional modification of gene expression may alter levels of MMP in RA and PsA, but studies of MMP levels in serum and synovial fluid do not provide evidence for an increase in MMP production in RA compared to PsA<sup>14,23,24</sup>. The location of the MMP-producing macrophages may be more critical to the development of erosion. MMP are predominantly produced in the lining layer of the synovium, which is thicker in RA compared to PsA<sup>35</sup>. A greater accumulation of MMP-secreting macrophages in the lining layer in RA may predispose to cartilage damage at the point of contact with cartilage<sup>36,37</sup>. Further studies are required to compare localization of MMP production at the PsA CPJ. A number of other MMP — in particular MMP-2 and MMP-13 — have also been implicated in the pathogenesis of RA and they may play a more critical role in mediating joint erosion<sup>38-40</sup>. It should also be noted that other cell types may be more important in the development of erosion at the CPJ. RA synovial tissue produces osteoclast-differentiating factor<sup>41</sup>, and there is emerging evidence that the osteoclast may be more important than the synoviocyte in the development of erosions at the CPJ in RA<sup>42,43</sup> and in the process of bony resorption in PsA<sup>44</sup>.

One criticism of this study is that the arthroscopic biopsy did not obtain synovium directly at the site of erosion. Synovial biopsies were obtained under direct vision at arthroscopy, which allowed synovium overlying the macroscopic CPJ of the knee to be scraped off the underlying cartilage. Studies of RA CPJ<sup>5,45,46</sup> suggest that the knee CPJ is not discrete but consists of a small area of transformed pannus, which includes the region that was biopsied. Synovial studies in RA have principally focused on the knee joint because it is possible to easily obtain adequate samples of knee synovium by either blind needle or arthroscopic biopsy<sup>36</sup>. But there is evidence that the CPJ in small joints, where the most distinctive differences in PsA and RA radiology are observed, is a “distinct” junction of cartilage and pannus<sup>42,45</sup>. This may explain the lack of correlation of radiological changes in the hands and feet of our patients with synovial MMP mRNA expression in the knee. The recent development of arthroscopic synovial biopsy of the metacarpophalangeal joints may allow the pathophysiologic mechanisms of small joint erosion to be addressed directly<sup>47</sup>.

Analysis of patients who had radiological damage at the time of synovial biopsy revealed that patients with erosions had higher numbers of clinically inflamed joints. Given that all these patients had RA, this may simply be a reflection of the higher number of inflamed joints present in RA. No significant relationship was observed between systemic indicators of inflammation or MMP mRNA expression and

the presence of radiological damage at the time of biopsy. Longitudinal radiological followup is under way to determine whether MMP expression at the CPJ predicts subsequent radiological damage in both the small joints of hands and feet and in the knee. Interestingly, there was an increase in the CPJ/non-CPJ ratio of TIMP-1 in nonerosive patients, although this did not quite reach statistical significance. This study confirms that MMP-1, MMP-3, and TIMP-1 gene expression is equally increased in both RA and PsA synovitis. The question facing rheumatologists is why RA and PsA synovitis produce disparate radiological outcomes.

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