Synovial fluid and serum concentrations of aminoterminal propeptide of type III procollagen in healthy volunteers and patients with joint disease

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Abstract

***Objectives*—To analyse synovial fluid and serum concentrations of the aminopropeptide of the type III procollagen (PIIINP) in normal individuals and patients with joint disease, and to explore the relationship between synovial fluid PIIINP concentrations and the rheumatological diagnosis, local inflammation, and joint disease.**

***Methods*—A radioimmunoassay was used to measure the PIIINP concentrations in serum and knee joint synovial fluid from 16 healthy volunteers and patients with osteoarthritis (OA) (n = 40), rheumatoid arthritis (RA) (n = 30), and psoriatic arthritis (PsA) (n = 12). The PIIINP measurements were related to demographic data, synovial fluid leucocyte counts, and radiograpic changes at the knee.**

***Results*—Serum PIIINP concentrations were greater in each of the disease groups than in control subjects, but there were no differences between the disease groups. Synovial fluid concentrations of PIIINP were much greater than those in serum, indicating local production, and were significantly greater in RA than in other disease groups ($p < 0.001$). There was only a weak positive correlation between synovial fluid leucocyte counts, some radiographic changes, and synovial fluid PIIINP concentrations.**

***Conclusions*—These data suggest that synovial fluid PIIINP concentrations may reflect local synovial proliferative processes in joint disease, and that they could be of diagnostic and prognostic value in inflammatory arthropathies.**

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The normal synovial membrane is composed of synovial cells supported by a loose fibrillar network, consisting primarily of a mixture of fibres derived from types I and III collagens.[1–3] In joint diseases the membrane becomes inflamed. However, the degree of inflammation (synovitis) varies, and is much greater in some conditions (for example rheumatoid arthritis (RA) and psoriatic arthritis (PsA)) than in others (for example osteoarthritis (OA)). Moreover, the nature of synovial response may vary with differing degrees of synovial proliferation. In RA, this proliferative response is more marked than in other disorders of the joints and is associated with an enhanced synthesis and deposition of type III collagen in the rheumatoid synovial tissue.[4]

Type III collagen is primarily synthesised during growth and development, wound healing and inflammation, and is one of the main fibrillar collagens present in the synovium. Type III collagen is synthesised as a procollagen molecule with non-collagenous aminopropeptide (PIIINP) and carboxypropeptide (PIIICP) extensions that are secreted into the extracellular space and can be measured in both serum and synovial fluid. Both PIIINP and PIIICP concentrations therefore reflect the rate of type III collagen synthesis.[5]

Serum concentrations of PIIINP have been studied widely;[6–9] they are believed to provide prognostic information[6 10] in RA, and may provide some information on disease activity in scleroderma. Synovial fluid concentrations are much less studied, but may reflect local production by proliferating synovium.[5 6] The present study was designed to test the hypothesis that synovial fluid concentrations of PIIINP would differ in the different types of rheumatic disease because of variations in the synovial proliferative response. Serum and synovial fluid PIIINP concentrations were assayed in control subjects and different disease groups, and related to measures of local inflammation and radiological change in order to assess whether the release of the epitope is simply a measure of the degree of synovitis or joint damage, rather than reflecting a more specific aspect of the disease process, such as synovial proliferation, which is difficult to assess with other techniques.

Patients and methods

SUBJECTS

Local ethics committee approval was obtained for the study. Patients entered into the study were those referred to the Bristol Royal Infirmary Rheumatology outpatient clinics who had painful knees and acute arthritis requiring synovial fluid aspiration from one or both knees for therapeutic reasons. The diagnosis of OA was based on the combination of use related pain and radiographic changes of definite OA (Kellgren and Lawrence grade 2 or

more,[11] RA using American Rheumatism Association criteria,[12] and psoriasis by the criteria of Baker *et al.*[13] There were 40 patients with OA of the knee (20 paired serum and synovial fluid), 30 with RA (13 paired), and 12 with PsA (nine paired). None of the study patients with OA or PsA had received steroid or disease modifying drugs for at least three months before entering the study: they were taking non-steroidal anti-inflammatory drugs (NSAIDs) or mild analgesic only. However, four of the patients with RA were receiving second line agents. Sixteen (all paired) healthy individuals with no knee pain and no history or signs of joint disease (normals) were used in the study. Healthy volunteers provided written informed consent for aspiration of synovial fluid from one knee joint.

METHODS

Synovial fluid from the knee joint was aspirated from the suprapatellar pouch from a lateral approach using a 23 gauge needle, examined within two hours by light microscopy and the total leucocyte count recorded. The fluid was then centrifuged at 4000 rpm for 10 minutes to remove cells and debris, aliquotted and stored at −70°C. Blood samples collected were allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes. Aliquots were stored frozen at −70°C. All aliquots of serum and synovial fluid used in the study were only thawed once immediately before assay.

ASSESSMENT OF RADIOGRAPHS

No radiographs were taken of the knee joints of healthy volunteers; those of the knee joints of the patients were taken on the same day or within three months of the aspiration of synovial fluid. Standing anteroposterior knee radiographs in full extension and supine lateral views in 30° flexion were obtained. Radiographs were scored in a fashion similar to that described by Cooper *et al*[14] by a single observer (EG) who was blinded to the clinical and biochemical data. Radiographic features recorded from each compartment (medial, lateral and patellofemoral) included osteophyte (0 = none, 1 = present, 2 = severe), subchondral bone sclerosis (present or absent), and erosions (present or absent). Joint space narrowing was recorded as present or absent in the patellofemoral joint and was measured in millimetres using a straight rule, at the mid point of the medial and lateral tibiofemoral joint.

N-PROPEPTIDE OF THE TYPE III PROCOLLAGEN (PIIINP)

A radioimmunoassay (RIA-gnost PIIIP, from Behring, Behringwerke AG, Germany) that uses human standards was used to measure the PIIINP concentrations in serum and knee joint synovial fluids. This assay preferentially recognises the intact newly synthesised peptide and is insensitive to smaller degradation products.[9] All serum and synovial fluid samples were assayed in duplicate, either undiluted (serum) or diluted 1:10 (SF). The intra- and interassay variations of the assays for serum were 5% and 8%, respectively, and those for synovial fluid were 6% and 13%, respectively.

STATISTICAL METHODS

Linear regression analysis was used to test for association between PIIINP concentrations, age, and disease duration. Comparisons were made using one way analysis of variance. To test for between group differences in the concentrations of PIIINP, post hoc pairwise comparisons were made using Tukey's simultaneous confidence intervals. The association between serum and synovial fluid PIIINP concentrations and radiographic features were tested, having allowed for disease group, using two way analysis of variance; correlations were calculated using the Spearman correlation coefficient. Statistical significance was set at the 5% level.

Results

Table 1 shows the demographic data of all the subjects in the study. The groups were not matched for age or gender, and there were significant differences in mean age, gender, and disease duration of the patients in all three disease groups.

Table 2 shows the serum concentrations of PIIINP. All three disease groups had greater serum PIIINP concentrations than the normal controls ($p < 0.001$ for OA and RA; $p = 0.079$ for PsA), but there were no significant differences between the disease groups. Regression analysis (allowing for disease groups) showed no association between serum concentrations of PIIINP and age ($p = 0.925$), gender ($p = 0.430$) or disease duration ($p = 0.990$).

The volume of synovial fluid aspirated did not affect the concentrations of PIIINP (fig 1). The mean synovial fluid concentrations of PIIINP were much greater than those in serum in all groups, including the normal subjects (table 2). There were no significant correlations between the serum and synovial fluid PIIINP concentrations ($r = 0.246$), $p = 0.063$, $n = 58$). Calculation of the median synovial fluid:serum PIIINP ratios for the paired samples showed that the ratios were in the order: normal controls < OA < PsA < RA (table 2). Figure 2 shows the individual synovial fluid PIIINP concentrations in each group. Synovial fluid concentrations were greatly increased ($p < 0.001$) in all three disease groups compared with the normal

Table 1 Demographic data of the normal subjects and patients studied

	No. of subjects	*No. of knees*	*Sex M/F*	*Age (yr)*†	*Disease duration (yr)*†
Normal	16	16	13/3	39·7 (23–51)	—
OA	40	41	10/30	64·6 (48–89)	13·9 (1–39)
RA	30	36	7/23	56·4 (19–74)	8·3 (11–23)
PsA	12	16	10/2	50·6 (23–72)	7·5 (0–13)

†Mean (range).

Table 2 PIIINP concentrations in paired synovial fluid (SF) and serum samples from normal controls and patients with inflammatory and non-inflammatory arthritis

	SF (U/ml)	*Serum (U/ml)*	*SF:serum ratio*
Normals (n = 16)	1·25 (0·60–3·70) 16	0·30 (0·20–0·60) 16	3·50 (1·23–8·00) 16
OA (n = 20)	3·53 (0·99–15·36) 20	0·52 (0·35–0·83) 20	3·80 (1·03–8·93) 20
RA (n = 13)	45·50 (4·9–168·40) 13	0·50 (0·35–0·86) 13	8·96 (1·30–260·00) 13
PsA (n = 9)	6·79 (2·93–80·49) 9	0·39 (0·30–1·18) 9	4·58 (1·25–8·27) 9

Values are median (range).

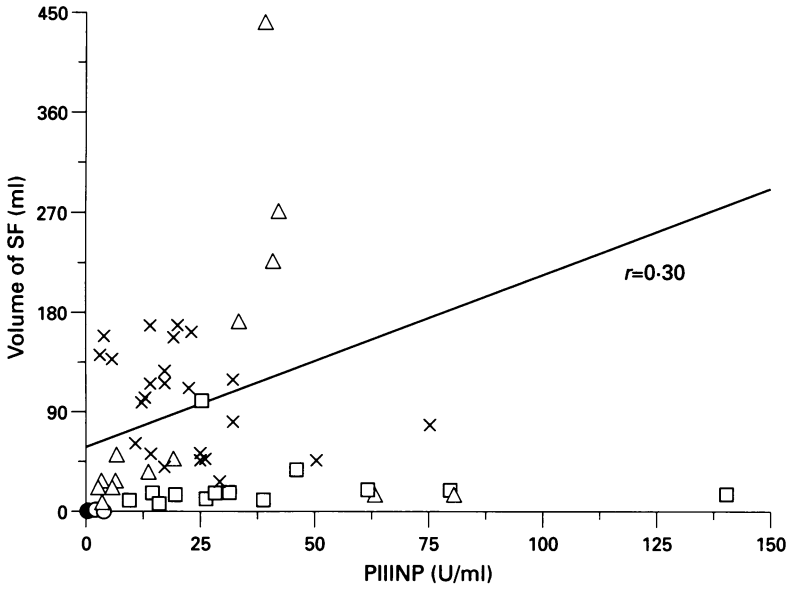

Figure 1 Relationship between concentrations of aminoterminal propeptide of type III procollagen (PIIINP) in synovial fluid (SF) and total volume of fluid aspirated. ○ = Healthy volunteers (n = 16); □ = osteoarthritis (n = 13); × = rheumatoid arthritis (n = 24); △ = psoriatic arthritis (n = 14).

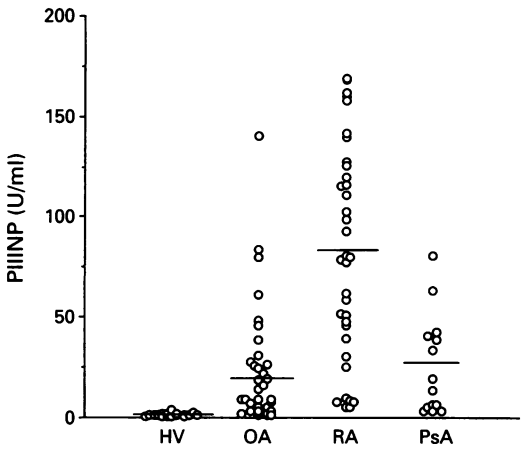

Figure 2 Synovial fluid concentrations of the aminoterminal propeptide of type III procollagen (PIIINP) in healthy volunteers (HV; n = 16) and patients with inflammatory and non-inflammatory joint disease. OA = Osteoarthritis (n = 40); RA = rheumatoid arthritis (n = 30); PsA = psoriatic arthritis (n = 12). The horizontal bars indicate the mean for each group.

controls. In addition, while the PIIINP values were very similar in synovial fluids of patients with OA and PsA, they were significantly greater ($p < 0·001$) in RA patients compared with the other disease groups (fig 2, table 2). The synovial fluid concentrations of the collagen propeptide showed a significant positive association with age ($r = 0·316$, $p = 0·005$, Ancova), but was unaffected by gender ($p = 0·535$) or disease duration ($p = 0·176$).

Table 3 Synovial fluid total leucocyte counts (× 10^5/ml) in normal subjects and patients with inflammatory and non-inflammatory arthritis

	Normals (n = 8)	*OA* (n = 31)*	*RA (n = 34)*	*PsA (n = 11)*
Median	0·14	0·7	22·5	13·0
Range	(0·06–0·84)	(0·21–1·30)	(1–100)	(2–200)

*Values are rough estimates: accurate counts not possible because of clumping of cells.

To examine if the synovial fluid total leucocyte count reflected local inflammation in joint diseases and was associated with locally produced PIIINP, the leucocyte counts of the different groups were compared with each other and with synovial fluid PIIINP concentrations. The counts were greater in all the disease groups compared with the normal controls (table 3) and, as expected, the inflammatory groups had greater leucocyte counts than the non-inflammatory group. The leucocyte counts in OA synovial fluid were estimates rather than accurate counts and therefore were not used in any calculations. When the counts of the remaining groups were put together and correlated with synovial fluid PIIINP concentrations, a weak positive correlation ($r = 0·453$, $p < 0·001$) was found. After allowing for disease group, this correlation was no longer significant ($r = -0·148$, $p = 0·089$).

To test for any possible associations between joint damage and PIIINP concentrations, various radiographic features of the knee joint were related to the concentrations of PIIINP measured in the synovial fluids from the same joint in addition to those in serum. As the distribution of PIIINP concentrations was found to be highly skewed (non-normal distribution), logarithmically transformed (natural log or Ln) data were used for analysis, but no significant association was found between serum PIIINP and any of the radiographic features. Table 4 shows the corresponding associations for synovial fluid. There was no association between osteophyte formation and synovial fluid PIIINP concentrations, but patients with sclerosis or joint space narrowing in the patellofemoral compartment had significantly greater synovial fluid PIIINP concentrations than those without these features. In addition, RA patients with erosions tended to have greater synovial fluid concentrations of PIIINP compared with those without erosion (not statistically significant) (table 4).

Discussion

We studied PIIINP in paired serum/synovial fluid samples from healthy volunteers and representative groups of patients with OA, RA, and PsA. We believe this to be the first comprehensive study of PIIINP concentrations in paired samples from healthy volunteers and patients with joint disease. The main objective of the study was to relate synovial fluid concentrations of the collagen propeptide to the degree of local inflammation (leucocyte count in synovial fluid) and joint damage, and to test the hypothesis that synovial fluid PIIINP concentrations reflect a separate aspect of the

Table 4 Associations between radiographic features and synovial fluid PIIINP concentrations (log transformed) in patients with inflammatory and non-inflammatory arthritis

Radiographic feature	*Scoring*	*n*	*Ln PIIINP†*	*p*
Osteophytes				
Medial	0	31	2·943 (1·466)	
	1	34	3·676 (1·199)	
	2	13	2·665 (1·735)	NS
Lateral	0	19	3·544 (1·230)	
	1	38	3·206 (1·470)	
	2	21	2·939 (1·595)	NS
PFJ	0	14	3·727 (1·376)	
	1	48	3·194 (1·498)	
	2	15	2·880 (1·341)	NS
Sclerosis				
Medial	Absent	54	3·303 (1·140)	
	Present	23	3·104 (1·524)	NS
Lateral	Absent	42	3·105 (1·414)	
	Present	35	3·410 (1·469)	NS
PFJ	Absent	44	2·993 (1·515)	
	Present	33	3·546 (1·324)	0·002
Erosions				
Medial	Absent	74	3·127 (1·434)	
	Present	4	4·865 (0·218)	0·170
Lateral	Absent	77	3·210 (1·458)	
	Present	1	3·671 (–)	
PFJ	Absent	76	3·205 (1·448)	
	Present	1	5·072 (–)	
Joint space narrowing				
PFJ	Absent	25	3·783 (0·918)	
	Present	32	4·005 (0·871)	0·027
Medial	(mm)	76	Correlation $r = -0·198$‡	NS
Lateral	(mm)	76	Correlation $r = -0·498$‡	NS

†Mean (SD). ‡Spearman rank correlation coefficient between joint space narrowing measured in millimetres (continuous variable) and synovial fluid PIIINP levels.
PFJ = Patellofemoral joint.

disease process in a joint. Our results show that serum and synovial fluid PIIINP concentrations were increased in patients with arthritic conditions compared with normal controls, suggesting that increased type III collagen propetide turnover is a characteristic of these joint diseases. However, there were no significant associations with synovial fluid PIIINP concentrations and evidence of local inflammation, and only a weak association with some of the radiographic changes.

Serum PIIINP concentrations have been studied widely in RA[6–9] and are generally high in patients with RA compared with healthy controls,[15] correlate with disease activity, and thought to be a useful prognostic marker in RA.[6 10] One study found increased serum PIIINP concentrations in a small group of RA patients with erosive progression,[6] but in the present study serum concentrations were not related to radiographic changes in the knee joint aspirated. As serum PIIINP is subject to many systemic influences, and only a small fraction is likely to be derived from the one arthritic joint aspirated, it is difficult to draw any conclusions about the value of serum PIIINP as a marker of local joint pathology.

The synovial fluid PIIINP concentrations were many times greater than those in serum in the disease groups and in the controls, suggesting that there is major local production of PIIINP in the joint and that the synovial fluid concentrations mainly reflect local synthesis. Recent studies demonstrated the presence of type III collagen in bone[16] and cartilage.[17 18] The greater synovial fluid PIIINP concentrations in patients with joint disease may therefore be attributable to a mixture of release of PIIINP from bone and cartilage, and synthesis in the synovium. The weak relationships between more severe radiographic changes and greater synovial fluid PIIINP concentrations suggest a possible association with more severe joint damage, but it seems likely that synovium is the major contributor to the increased synovial fluid concentrations.

Active proliferation of connective tissue results in high serum concentrations of PIIINP and the increased values are believed to result from de novo synthesis in the inflamed synovium.[7 10] Thus it is not surprising to find increased concentrations of PIIINP in synovial fluid from inflammatory RA compared with non-inflammatory OA or normal controls, and in this regard our results confirm those of Horselev-Petersen *et al.*[9] However, we have made a new and intriguing observation that, despite evidence of greater synovial inflammation, synovial fluid concentrations of PIIINP were significantly ($p < 0·001$) less in patients with PsA than in those with RA, and similar to values in those with OA. This suggests that there may be fundamental differences in the disease process of these two inflammatory arthritides—a view that is further supported by the finding of a significant difference between the synovial fluid/serum ratios of PIIINP in RA and PsA. The mechanisms controlling the production of synovial fluid PIIINP may differ in different diseases. The highly significant difference ($p < 0·001$) between RA and PsA may reflect increased fibroblastic activity in RA, contribution from other tissues within the RA joint, or both.

The differences in the serum and synovial fluid concentrations of PIIINP between the normal controls and the arthritic groups, or between the arthritic groups themselves, could have resulted partly from background variables such as age, gender and disease duration: indeed, synovial fluid PIIINP was found to be positively associated with age. However, we were able to account for such variables using the linear regression model. Also the volume of synovial fluid aspirated may affect the concentrations of PIIINP—that is to say, the larger volumes of synovial fluid in PsA compared with RA could account for the smaller concentrations in the former group. However, we found that the volume of fluid aspirated was not related to PIIINP concentrations. Another possible confounding variable that could affect PIIINP concentrations is drug treatment: azathioprine, for example, is known to suppress serum PIIINP in active RA.[7 9] None of the patients with OA or PsA in the present study received steroid or second line agents for at least three months before joint aspiration and serum collection. In contrast, some RA patients did receive disease modifying drugs that would be expected to reduce, not increase, PIIINP concentrations in synovial fluid.

Several earlier reports associated serum PIIINP concentrations with disease progression in RA.[6 10] As the synovial fluid concentrations of PIIINP is highly increased in RA, while the serum concentration was found to be similar to that in the other arthritic groups

in this study, the synovial fluid concentrations of PIIINP might be of even greater prognostic value than serum concentrations, for an individual RA joint. Moreover, the highly significant increase in synovial fluid PIIINP concentrations in RA compared with PsA suggests that the synovial response is different in the two disorders. In early seronegative arthritis, it may be difficult to differentiate seronegative spondarthritides from early RA, in which most of the conventional serum or synovial fluid assays are often non-discriminatory. Synovial fluid PIIINP concentrations may provide a helpful diagnostic marker in these conditions, but these results require confirmation in a study of larger numbers of patients, and the potential prognostic value of synovial fluid PIIINP concentrations requires investigation in a prospective study.

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