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Association between synovial fluid levels of inorganic pyrophosphate and short term radiographic outcome of knee osteoarthritis

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Abstract

***Objective*—To test the hypothesis that high concentrations of extracellular inorganic pyrophosphate (PPi), which associate with increased cell synthesis and turnover in cartilage, may act as a marker for structural outcome in knee osteoarthritis (OA).**

***Method*—One hundred and thirty five consecutive patients referred to hospital with knee OA (59 men, 76 women; mean age 71 years, range 41–88) were followed prospectively for a median of 2·5 years (interquartile range 1·75–3·0). Synovial fluid (SF) aspirated at presentation (202 OA knees: 68 bilateral, 66 unilateral) was assessed for PPi content by radiometric assay. Knee radiographs at presentation and at final review were assessed for change in global (Kellgren) and individual features (narrowing, osteophyte, sclerosis, cyst, attrition) of OA.**

***Results*—The median SF PPi level was 10·5 μmol (range 0·07–72·4). At baseline, high PPi was significantly associated with presence of calcium pyrophosphate crystals, chondrocalcinosis, and bone attrition. Radiographic change was observed in 164 knees. High PPi levels were negatively associated with change in Kellgren and Lawrence grade, further narrowing, and increase in osteophyte, but positively associated with development of attrition. In the 68 patients from whom bilateral data were obtained, there was correlation between right and left knees for PPi levels, all baseline radiographic scores, and changes in radiographic features. Multiple logistic regression analysis for PPi as a continuous variable (age, gender, and patient number included in model) showed a negative correlation with change in global Kellgren and Lawrence grade (odds ratio (OR) 0·97, 95% confidence interval (CI) 0·95 to 0·99) and a positive correlation with attrition (OR 1·04, 95% CI 1·02 to 1·07).**

***Conclusion*—High SF levels of PPi are associated with favourable radiographic outcome in terms of progressive change in Kellgren grade. Such elevated PPi levels, however, may inhibit new bone formation and remodelling in knee OA.**

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Inorganic pyrophosphate (PPi) is a by product of many essential biosynthetic reactions in mammalian cells.[1] There is no recognised transmembrane transport mechanism for PPi, and two mechanisms are postulated to contribute to extracellular PPi levels: first, leakage of intracellular PPi or coextrusion with other cellular products;[1] second, and more importantly, salvage degradation of extracellular ATP by ectoenzymes, including nucleotide triphosphate pyrophosphohydrolase.[1-8] Increased cellular activity and division may lead to elevated extracellular concentrations of PPi by either mechanism.[1]

In articular tissues, the chondrocyte and its associated vesicles are considered the principal source of extracellular PPi.[1-4 8] Concentrations of PPi in knee synovial fluid (SF) are increased in osteoarthritis (OA) and in OA associated with calcium pyrophosphate dihydrate (CPPD) crystal deposition ('chronic pyrophosphate arthropathy').[5 7] Both these conditions are characterised by increased metabolic activity in cartilage and by new bone formation.[9] In contrast, subnormal levels of SF PPi occur in knees affected by rheumatoid arthritis,[7] a condition associated with marked attrition of cartilage and bone. Such observations suggest the possibility that increased concentrations of PPi resulting from increased biosynthetic activity may act as a marker for a potentially 'hypertrophic' (as opposed to 'atrophic') response to articular insult.[7 9] If this were true, SF PPi levels might be expected to correlate with structural change and radiographic outcome in OA. This study was undertaken to test this hypothesis.

Patients and methods

The study was approved by the local research ethics committee. Consecutive patients referred with symptomatic knee OA to one general rheumatology clinic were recruited over a two year period. Details of these patients, part of a larger cohort, have been described previously.[10 11] Alternative arthropathies were excluded by clinical examination, blood tests (serology, acute phase response), microscopy of SF, and radiographic screening.[11] Knee OA was defined radiographically, from standing fully extended anteroposterior and 30° flexion lateral views, as presence of joint space narrowing with osteophyte, sclerosis, or both, in at least one knee compartment (patellofemoral, medial tibiofemoral, lateral tibiofemoral).

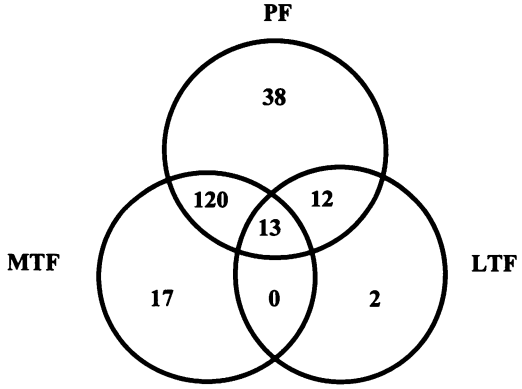
ASSAY OF SYNOVIAL FLUID INORGANIC PYROPHOSPHATE

All OA knees were aspirated at presentation, irrespective of whether an effusion was detected clinically. A sample of each SF was examined fresh, by compensated polarised light microscopy, for presence of CPPD crystals (identified by characteristic morphology and birefringence).

For the PPi assay, SF was taken into sterile plastic containers on ice, spun at 2500 *g* for 15 minutes at 4°C, and the resulting supernatant stored frozen at −20°C. SF PPi levels were estimated using a modification[7] of a specific, sensitive radiometric assay.[12] In brief, the SF sample PPi was allowed to react with tritiated uridine diphosphogluconate, and the product, glucose-1-phosphate, was converted to a labelled, stable, recoverable product (tritiated 6-phosphogluconate) estimated by radioactivity count (each sample assayed in triplicate). To measure any hydrolysis of PPi to orthophosphate during incubation and extraction, a recovery procedure was undertaken (performed in duplicate) involving addition of tracer [^{32}P]PPi to the initial sample, removal of orthophosphate by complexing with ammonium molybdate and recovery into isobutanol/light petroleum, and counting of the remaining [^{32}P]PPi in the aqueous phase.

RADIOGRAPHIC ASSESSMENTS

Standardised plain radiographs of knees were obtained at the patient's entry to the study and then at least annually. Study films were a standing anteroposterior radiograph of the tibiofemoral joints (55 kV, 8 mA s, full scale deflection (FSD) 100 cm) plus lateral views in 30° flexion of the patellofemoral joint (55 kV, 8 mA s, FSD 100 cm). Initial films were examined and scored by two observers as described previously.[10 11] Each knee compartment was given a global radiographic score for OA using a system based on the Kellgren and Lawrence scale.[13] In addition, individual features of OA (narrowing, osteophyte, sclerosis, cysts) were graded on a scale of 0–3 using a modification of the method described by Thomas *et al.*[14] Chondrocalcinosis (calcification in hyaline or fibrocartilage) and attrition (loss/collapse of subchondral bone stock resulting in apparent reduced bone volume) were each recorded as present or absent. The last available knee radiographs were assessed in a manner identical to that applied to the study entry film (blinded to the original assessment). Individual radiographic features (narrowing, osteophyte, cysts, sclerosis, attrition) were assessed for change (increase, same, decrease) at each knee compartment, by comparison of scores from initial and final radiographs. Reproducibility (for the same assessors) for the scoring of individual radiographic features and Kellgren grade, and for the scoring of change in individual radiographic features had previously been demonstrated to be good.[10 11]



Compartmental distribution of OA change in the 202 study knees from which synovial fluid for PPi estimation was obtained. PF = Patellofemoral; MTF = medial tibiofemoral; LTF = lateral tibiofemoral.

STATISTICAL ANALYSIS

Statistical analysis was by the Mann Whitney *U* test, logistic regression (Egret, Serc, Seattle), Spearman rank correlation, and the kappa (κ) statistic as appropriate. The κ statistic was used as a measure of association—that is, as a test of symmetry over and above that expected by chance. Correlation between results from right and left knees in those with bilateral data was undertaken on Statpak using the correlation and regression programme; because correlations were found, a dummy patient variable was included in the regression model to take this into account.

Results

One hundred and thirty five consecutive patients (76 women, 59 men; mean age 71 years, range 41–88) were recruited. Knee OA was bilateral in 117 (87%). An amount of SF sufficient for estimation of PPi levels was obtained from 202 (81%) OA knees (134 patients)—specifically, from both knees of 68 patients and from one knee of 66 patients.

RADIOGRAPHIC FINDINGS

The figure shows the compartmental distribution of OA change (any of the specified OA features) for the 202 study knees at entry, and table 1 lists the mean summated (medial and lateral tibiofemoral, patellofemoral compartments) radiographic scores at entry for the various measures. Chondrocalcinosis was present in 70 (34%) knees (43 patients), and CPPD crystals were identified in 68 (33%) knees (40 patients).

In the 68 patients for whom bilateral knee SF data were obtained, there was close correlation between radiographic findings at baseline in

Table 1 Summated radiographic scores (patellofemoral and lateral and medial tibiofemoral compartments) for various features

Radiographic feature	*Mean*	*Median*	*Range*
Kellgren & Lawrence grade	3·67	4	1–8
Narrowing	3·67	4	1–8
Osteophyte	4·08	4	0–9
Cysts	1·12	1	0–6
Sclerosis	1·70	2	0–6
Attrition	0·75	0	0–4

Table 2 Correlation between right and left knees for radiographic features at baseline and for change with time in 68 patients for whom there were bilateral knee data

Radiographic feature correlation	Baseline	Change with time
	r^2	r^2
Summated (total knee) Kellgren grade	0·68	0·43
Summated patellofemoral score	0·73	0·38
Summated medial tibiofemoral score	0·69	0·38
Summated lateral tibiofemoral score	0·62	0·83
Summated (three compartment) score for:		
Narrowing	0·68	0·50
Osteophyte	0·73	0·28
Cyst	0·65	0·30
Sclerosis	0·44	0·57
Attrition	0·70	0·47

right and left knees with respect to compartmental distribution, summated Kellgren scores and scores for individual features (table 2).

The median duration of follow up was 2·5 years (interquartile range 1·75–3·0). Over this period, 164 knees showed change in at least one radiographic feature, and 38 knees showed no change. Table 3 shows the frequency of change in each radiographic feature during the study. In the 68 patients with bilateral data, there was close agreement between right and left knees for change in each radiographic feature (table 2); for example, for increase in Kellgren grade the correlation was 0·43, with a κ of 0·71, and odds ratio of 6·75 (95% CI 1·97 to 24·24).

SPECIFICITY, SENSITIVITY AND VARIABILITY OF PPi ASSAY

The limit of detection of the PPi assay was 100 pmol of PPi. Specificity was demonstrated by significant loss of radioactivity on addition of yeast pyrophosphatase to the final incubation. Recovery of PPi added to SF (in triplicate measurements) ranged from 92% to 105% when concentrations of added PPi ranged from 0·5 to 8 μmol. The coefficient of variation for the assays was 9% (same day estimations on 10 aliquots of the same SF), and the mean coefficient of variation ($n = 10$) between triplicate measurements was 8%.

CROSS SECTIONAL DATA AT PRESENTATION

A wide range of values of SF PPi was found (median 10·5 μmol, range 0·07–72·40). In the 68 patients for whom there were bilateral SF data, there was correlation between levels in right and left knees ($r^2 = 0·84$). Levels were significantly greater in women than in men (median 13·0 μmol (95% CI 9·3 to 14·8) compared with 7·2 μmol (95% CI 6·6 to 9·8), respectively) ($p < 0·01$) and increased significantly with increasing age of the subject (median 9·1 μmol (95% CI 7·2 to 12·8) for age <80 years, compared with 14·3 μmol (95% CI 11·9 to 16·3) for age >80 years) ($p < 0·01$). High levels of PPi were associated with presence of CPPD crystals (median levels for presence and absence 18·5 μmol (95% CI 15·8 to 25·0) compared with 7·4 μmol (95% CI 6·3 to 9·3), respectively), chondrocalcinosis (median 16·0 μmol (95% CI 13·4 to 24·7) compared with 8·0 μmol (95% CI 6·6 to 10·7)), and bone attrition (median 14·8 μmol (95% CI 13·0 to 18·6) compared with 7·8 μmol (95% CI 6·6 to 9·6)) ($p < 0·001$). No association was found with presence of joint space loss, osteophyte, sclerosis, or cyst formation.

Table 3 Frequency of change in radiographic features of knee osteoarthritis

Change in radiographic feature	No of knees	(%)
Further joint space narrowing	120	59
Increase in osteophyte	65	32
Increase in cyst	44	21
Increase in sclerosis	36	18
Development of attrition	44	22
Increase in Kellgren grade	86	46

PROSPECTIVE DATA

There was no difference in PPi levels between the 38 knees showing no radiographic change (median 11·3 μmol, 95% CI 7·2 to 15·6) and the 164 showing some change (median 10·4 μmol, 95% CI 7·7 to 13·7). However, associations were noted for changes in individual radiographic features. Lower levels of SF PPi associated with further decrease in joint space and change in osteophyte: median levels 8·8 μmol (95% CI 7·2 to 12·4) compared with 12·6 μmol (95% CI 7·8 to 14·7) ($p < 0·01$), and 7·2 μmol (95% CI 5·9 to 9·1) compared with 12·7 μmol (95% CI 9·1 to 15·0) ($p < 0·001$) in knees with and without change, respectively.

Table 4 shows results for change in Kellgren grade and change in individual radiographic features when PPi was examined as a continuous variable, and when PPi levels were grouped as quartiles, for which the ranges of values were: 0·07–5·5 μmol (1st quartile); 5·6–10·5 μmol (2nd quartile); 10·6–16·9 μmol (3rd quartile); 17·0–72·4 μmol (4th quartile).

High levels of PPi associated negatively with increase in Kellgren scores and increase in osteophyte, but positively with development of attrition. No linear association between PPi levels and change in either narrowing or cyst was discerned; for these two features both very low (1st quartile) and very high (4th quartile) PPi levels showed greatest tendency to change. Low levels of PPi associated with change in sclerosis. However, when logistic regression was used to examine PPi as a continuous variable, with age, gender, and 'dummy' patient variable included in the model, two of these associations remained: the negative association with change in Kellgren score (odds ratio (OR) 0·97, 95% CI 0·95 to 0·99), and the positive association with development of attrition (OR 1·04, 95% CI 1·02 to 1·07). A negative association between age and change in osteophyte (OR 0·97, 95% CI 0·94 to 0·99), but a positive association between age and attrition (OR 1·05, 95% CI 1·04 to 1·09) were also observed. No associations were found between PPi and change in osteophyte, cyst, or sclerosis.

Discussion

There is considerable interest in putative 'markers' of OA, with most attention focusing on components of cartilage, synovium, or bone

Table 4 PPi levels and change in radiographic features

	% with change	*χ^2 test for trend*	*p*	*Odds ratio*	*95% CI*
Kellgren & Lawrence grade					
PPi (continuous variable)				0·97	0·95 to 0·99
PPi quartiles		4·31	0·038		
1st	29			1·0	
2nd	29			1·09	0·46 to 2·59
3rd	22			0·54	0·23 to 1·28
4th	20			0·52	0·21 to 1·24
Osteophyte					
PPi (continuous variable)				0·99	0·96 to 1·01
PPi quartiles		9·19	0·002		
1st	35			1·0	
2nd	31			0·84	0·35 to 2·01
3rd	15			0·27	0·10 to 0·70
4th	18			0·37	0·14 to 0·94
Attrition					
PPi (continuous variable)				1·04	1·02 to 1·07
PPi quartiles		8·37	0·003		
1st	14			1·0	
2nd	18			1·47	0·41 to 5·30
3rd	27			2·10	0·65 to 6·98
4th	41			4·13	1·34 to 13·2
Narrowing					
PPi (continuous variable)				1·02	0·99 to 1·04
PPi quartiles		1·72	0·19		
1st	25			1·0	
2nd	20			0·67	0·28 to 1·60
3rd	25			0·83	0·35 to 1·96
4th	30			1·71	0·68 to 4·32
Cysts					
PPi (continuous variable)				1·02	
PPi quartiles		0·67	0·41		
1st	32			1·0	
2nd	11			0·30	0·08 to 1·01
3rd	18			0·45	0·15 to 1·30
4th	39			1·32	0·14 to 0·94
Sclerosis					
PPi (continuous variable)				0·99	0·96 to 1·01
PPi quartiles		4·97	0·03		
1st	38			1·0	
2nd	23			0·51	0·20 to 1·30
3rd	18			0·31	0·12 to 0·83
4th	21			0·44	0·17 to 1·12

CI = Confidence interval.

estimated in SF, serum, or urine.[15] This is the first prospective study of associations between SF PPi and radiographic change in knee OA. The rationale for investigating SF PPi as a variable of potential prognostic significance in OA was based on the following: extracellular PPi may reflect local cellular activity, damage, or both;[1-3 9] SF PPi predominantly arises from cartilage;[1-4 16] SF PPi is readily measurable using a sensitive and specific radiometric assay;[7 12] a variable increase in SF PPi above normal values has been demonstrated in knees with OA;[1 5-7] and SF PPi levels in normal knees do not increase or alter with age per se.[7 17]

The cross sectional data accord with previous studies in showing increased concentrations of SF PPi in OA,[5-7 17] and associations between high levels of PPi and presence of SF CPPD crystals and chondrocalcinosis.[7 17] The only radiographic feature of OA that we found to associate with high PPi levels was bone attrition. The prospective data showed high levels of PPi to associate also with development of attrition, whereas low levels associated with increase in global scores of OA. As far as we are aware, this is the first prospective study to show associations between an SF variable and subsequent radiographic change in OA.

There is no agreed method of assessing structural change or its progression in knee OA. Plain radiographs have several inherent problems, for example standardisation of positioning, magnification, and film quality, insensitivity for change, and problems with observer error. In this study we used standardised standing films to achieve better visualisation of loss of interosseous distance in tibiofemoral compartments,[14 18] and lateral flexion views for the patellofemoral compartment. Although we recently reported that the 'skyline' view has certain advantages over the lateral flexion view,[19] we were using lateral views alone at the start of this study. The same technique and film were used for all radiographs, and a large number of knees were studied. The global scoring system of Kellgren and Lawrence[13] is commonly used, but assumes that different features of OA progress together. Separate grading of individual features[14] is less commonly used, but may be more sensitive to change,[11] and assumes no particular hierarchy with respect to progression. We therefore used both scoring systems in this study, having shown good intra- and interobserver reproducibility for both systems in this same study population.[10 11] We found a negative association between PPi levels and change in Kellgren grade, but different (negative and positive) associations for individual features. This finding alone supports the need to consider individual features in outcome 'marker' studies. It may also explain in part the lack of difference between knees showing any change and knees with no change, the separate negative and positive associations tending to cancel out and reduce the group difference.

An interesting finding in the patients who contributed bilateral knee data was the positive correlation, both for radiographic scores and SF PPi levels, between right and left knees at baseline. Furthermore, the tendency to change in radiographic features in right and left knees also correlated. This lack of independence presents certain problems for analysis, which are only rarely considered in such studies. Clinically, there is interest in both knees with respect to outcome; exclusion of one knee (for example the least 'worst') for the purposes of statistical analysis therefore seems inappropriate. Having found evidence for dependency between right and left data in the same patient, we elected to correct for this by including a dummy patient-dependent variable in the logistic regression model. However, other methods of tackling this problem have been suggested recently.[20]

There are a number of important caveats to this study. First, we estimated SF PPi at a single time point and did not undertake serial estimations to assess fluctuations or trends over time. Second, because all patients had been referred to hospital, they may have represented a population with a more severe form of OA. Further study is required to establish to what extent these findings may be generalised to early, less severe OA. Third, these data relate to radiographic change alone. Multiple factors, including psychosocial ones, influence the severity of pain and disability in knee OA,[21 22] and control for such multiple factors was impractical for a study this size. The radiometric assay that we used for estimation of PPi is sensitive and specific, and can be applied to small SF volumes. However, the assay is complex and expensive in terms of materials and technician time. As with any SF assay, the

concentration measured depends on a number of variables including rate of synthesis, rate of dissolution, and SF clearance. We have shown previously that SF PPi levels in knee OA are not influenced by the degree of clinically assessed inflammation, though this can be an issue in OA knees with CPPD crystal deposition.[7] In the present study, greater SF levels of PPi occurred in women and in older subjects, perhaps reflecting more widespread compartmental involvement and tendency to CPPD deposition/chondrocalcinosis in these individuals. PPi levels may relate to deposition of both CPPD and calcium phosphate crystals, both of which commonly deposit in OA knees and have been suggested to associate with more severe, more widespread multicompartmental OA[10 23 24] and tendency to radiographic progression.[11] Whether such crystal deposition is cause or consequence of OA change, however, remains unclear. Crystal formation, growth, dissolution, and trafficking within joints are complex phenomena,[9] and identification and quantification of SF crystals are extremely problematic. Clear associations of calcium crystals with OA and SF PPi are therefore difficult to define.

PPi metabolism is incompletely understood, and these data are open to several interpretations. If cellular activity is an important influence tending, directly or indirectly, to increase extracellular levels of PPi,[1-9] then increased SF levels might be expected to associate with little progression of structural change ('compensated' OA). Conversely, low levels of SF PPi, reflecting poor cellular (predominantly chondrocyte) activity would be expected to associate with progression of structural change ('decompensated' OA). Extracellular concentrations of PPi can modulate cartilage and bone mineralisation, with moderate increase stimulating, but gross increase inhibiting, calcium phosphate crystal deposition.[1 25-28] Increased PPi levels that accompany exuberant cellular activity may therefore inhibit apatite deposition and bone remodelling, and thus associate with little change in osteophyte and a tendency to bone attrition. Of special interest was the finding that both very high and very low levels of PPi might associate with further joint space narrowing. This could be explained if very high levels result mainly from release of intracellular PPi as a result of cell death or damage.[1]

In summary, this prospective study of hospital referred patients with knee OA supports an association between SF concentrations of PPi at presentation and tendency to undergo further radiographic change over the next two to three years. Further investigation of SF PPi as a potential marker of the OA process seems warranted.

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