Proteoglycan fragments in joint fluid

Influence of arthrosis and inflammation

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We determined the concentration of proteoglycan fragments in knee joint fluid collected from knee-ligament injured patients more than 6 months after the trauma and from patients with acute pyrophosphate arthritis and arthrosis or with arthrosis only. Injured patients with normal or only mildly altered cartilage at arthroscopy and with normal radiographs, had twice the average concentration of healthy volunteers. Other injured patients with advanced, radiographic signs of arthrosis, had synovial fluid proteoglycan fragment concentrations within the range of healthy volunteers. Patients with pyrophosphate arthritis had the highest concentrations, substantially increased compared with both arthrosis patients, with or without knee injury and healthy volunteers. Likewise, there was an inverse relation between the degree of arthrosis and the concentration of proteoglycan fragments in the joint fluid in patients with pyrophosphate arthritis and arthrosis or with arthrosis only.

We conclude that synovial fluid levels of proteoglycan fragments are influenced by the mass of cartilage matrix remaining in the joint, the inflammatory activity in the joint, and the metabolic activity of the cartilage cells.

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Arthrosis has been defined by a combination of clinical and radiographic findings (Kellgren and Lawrence 1957, Ahlbäck 1968, Altman et al. 1986). However, when joint space narrowing becomes visible on plain radiographs, cartilage damage is already advanced. To facilitate an earlier diagnosis, to monitor the progress of the disease, and to evaluate surgical and pharmacologic treatments of arthrosis, we need improved diagnostic tools (Lohmander 1988, 1990, 1991). Fragments of cartilage-matrix molecules are released in the joint fluid, serum, and urine as a result of matrix turnover in both normal and diseased joint cartilage and may serve as such diagnostic tools.

Patients developing posttraumatic arthrosis may serve as a model for arthrosis in general (Johnson et al. 1974, Graham and Fairclough 1988, Holden et al. 1988, Sherman et al. 1988, Kannus and Järvinen 1989, Lohmander et al. 1989, Morrey 1989). These patients provide a relatively homogeneous subset of the otherwise heterogeneous arthrosis population. The time of the trauma is most likely the time of disease onset, and the arthrotic process can therefore be monitored from its earliest stage. It is also possible to obtain a precise diagnosis by arthroscopy. Lastly, the disease progression is comparatively rapid (Johnson et al. 1974, Graham and Fairclough 1988, Sherman et al. 1988, Kannus and Järvinen 1989, Morrey 1989).

We have shown that an acute injury of the cruciate ligament or meniscus of the knee causes a marked increase in the concentration of proteoglycan fragments in the joint fluid. Furthermore, patients studied several years after the trauma also have an abnormally high concentration as compared with an age-matched control group (Lohmander et al. 1989). Increased levels of cartilage markers in synovial fluid have also been demonstrated in patients with acute pyrophosphate arthritis (Ratcliffe et al. 1988, Lohmander et al. 1989) and in other inflammatory joint diseases (Saxne et al. 1986). There is, however, a wide range of concentration values within these groups of patients. To investigate the causes of this variability, we have now studied the influence of arthrosis and inflammation on the levels of proteoglycan fragments in knee synovial fluid. Some of these data have been published in a preliminary form (Lohmander et al. 1990, Lohmander and Dahlberg 1991).

Patients and methods

Patients

Synovial fluid was examined from five groups of patients—483 knees, one sample from each patient.

Group A 16 knee-healthy athletes;

Group B 123 patients with rupture of the cruciate ligament with or without associated meniscus tear or rupture to the collateral ligament. No patient in this group had had any knee-stabilizing surgery performed at the time of sampling;

Table 1. Grading of gonarthrosis. Groups 1–5 all had normal standing radiographs

Arthroscopy	
1	Normal cartilage by arthroscopy
2	Superficial fibrillation limited to one compartment
3	Superficial fibrillation in more than one compartment
4	Deep clefts to bone limited to one compartment
5	Deep clefts to bone in more than one compartment
Radiography, standing	
6	Subchondral sclerosis, minimal joint space change
7	Ahlbäck Stage I (> 50% reduction of cartilage height)
8	Ahlbäck Stage II (100% reduction of cartilage height)
9	Ahlbäck Stage III (bone erosion < 5 mm)
10	Ahlbäck Stage IV (bone erosion > 5 mm)

Group C 166 patients with a tear of the meniscus but no other diagnosed injury of the knee;

Group D 43 patients with acute pyrophosphate arthritis, as well as varying stages of arthrosis;

Group E 135 patients with arthrosis but no history of inflammatory episodes or of previous injury to the knee.

All patients were examined with radiography, the patients in groups B, C, D, and E with no radiographic signs of arthrosis were also examined by arthroscopy to confirm the diagnosis and to assess and grade the joint cartilage alterations. All the samples from the 289 knee-injured patients were obtained in a chronic phase more than 6 months after trauma. At the time of sampling, the knee-injured patients were older than 17 years. Patients were included in group D only if their history and clinical signs were strongly suggestive of acute pyrophosphate arthritis (pseudogout) and if microscopy of the current or a recent joint fluid sample demonstrated the presence of intracellular pyrophosphate crystals in the synovial-fluid cells (Bjelle 1988). The diagnosis, date of injury or duration of symptoms, sex and age of the individual patients were also recorded. None of the patients were treated with intraarticular steroids or were on chronic treatment with nonsteroidal antiinflammatory drugs.

Grading of arthrosis

The patients were graded on an arbitrary scale from 1 to 10 for joint cartilage changes (Table 1). The arthroscopic grading system, modified after Outerbridge (1961), evaluated the cartilage in the three knee compartments. The radiographs were assessed by the Ahlbäck method (1968). Patients with a score between 1 and 5 all had normal standing radiographs. Grading was assessed without knowledge of the results of the joint fluid analyses.

Joint fluid

The volume of the aspirated synovial fluid was measured and aliquots were taken for cell counting, polarizing microscopy (group D) and, when appropriate, for bacterial cultures to exclude septic arthritis (group D). 20 mL of 0.9 percent saline was then injected into the joint and aspirated after repeated passive flexion-extension or repeated compression of the infra- and suprapatellar regions (Geborek et al. 1988). The joint fluid and saline lavage samples were centrifuged and the supernatant stored frozen at –70 °C until analyzed (Lohmander et al. 1989).

The level of proteoglycan fragments in joint fluid and saline lavage samples was analyzed using an enzyme-linked immunosorbent assay (Heinegård et al. 1985, Saxne et al. 1986). The results are expressed as micrograms of intact joint-cartilage proteoglycan-monomer equivalents per mL of joint fluid.

Total joint cartilage mass

In order to quantify the mass of cartilage in a normal knee joint, the entire cartilaginous layer covering the joint surfaces was dissected from the underlying bone on autopsy of four healthy adult knees, less than 24 h post mortem. Cartilage was likewise dissected from five knees at the time of total joint replacement for arthrosis. The tissue slices were stored at –20 °C until extracted. Proteoglycan extraction of the lightly homogenized cartilage slices was done at 4 °C for 24 h with 15 mL of 4 M guanidine-HCl/g tissue, containing protease inhibitors (Lohmander et al. 1988). The proteoglycan content of representative aliquots was assayed by the orcinol procedure (Khym and Doherty 1952) and by a procedure based on precipitation of proteoglycans with Alcian blue (Björnsson, personal communication).

Statistics

Student's *t*-test (separate variances) or Wilcoxon's rank sum test were used for the statistical significance analysis. A probability value of less than 0.05 was considered significant.

Results

The reference Group A had an average concentration of 35 μg/mL of proteoglycan fragments in the joint

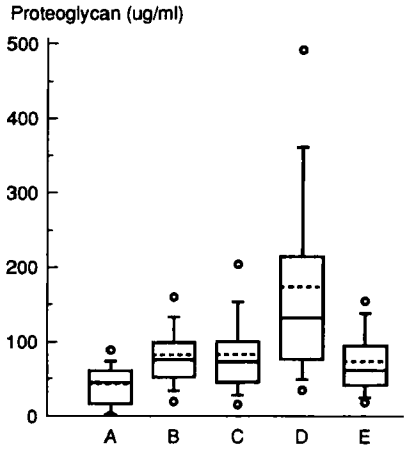

Figure. 1. The distribution of concentration values for proteoglycan fragments in joint fluid (μg/mL).

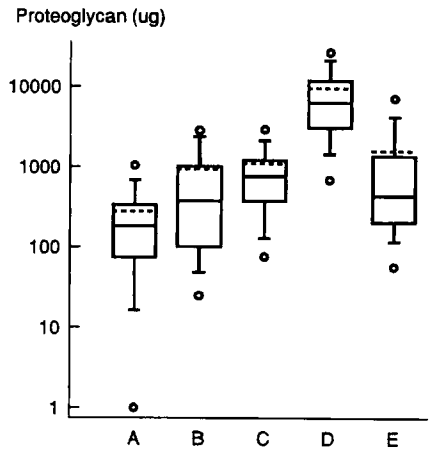

Figure 2. The distribution of total mass of proteoglycan fragments in joint fluid (μg).

The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively; the dividing line is the median value and the dashed line is the average value. The lower and upper limits of the whiskers represent the 10th and 90th percentiles, respectively. The open symbols represent the 5th and 95th percentiles, respectively.
A. Healthy athletes. B. Cruciate ligament lesion. C. Meniscus lesion. D. Pyrophosphate arthritis and arthrosis. E. Arthrosis only.

Table 2. Arthrosis score, number of patients (n), sex distribution (percent male), age at joint fluid sampling, duration of history (wk), volume of synovial fluid sampling, (mL), concentration of proteoglycan (μg/mL), total mass of proteoglycan aspirated (μg), in the five studied groups. Average and *SD*. For definition of A, B, C, D, E, see Patients and methods

Groups	Score	n	Sex	Age		Duration		Volume		Concentration		Total mass	
A		16	63	28	*7*			1	*1*	35	*27*	263	*332*
B	1–8	123	73	29	*9*	190	*174*	6	*14*	82	*46*	940	*796*
	1	68	72	31	*9*	150	*152*	3	*6*	85	*46*	630	*107*
	2	26	80	31	*9*	164	*111*	11	*24*	87	*58*	1557	*2931*
	3	10	60	33	*5*	233	*193*	4	*6*	77	*28*	570	*537*
	4	8	75	35	*8*	150	*114*	14	*13*	77	*48*	1995	*3337*
	5–6	7	57	39	*11*	430	*144*	11	*18*	47	*29*	1023	*672*
	7–8	4	100	56	*10*	547	*290*	7	*10*	84	*16*	1018	*1702*
C	1–8	166	77	38	*12*	179	*261*	6	*10*	83	*61*	1104	*1580*
	1	85	80	36	*11*	148	*214*	5	*9*	93	*72*	1155	*1831*
	2	30	73	44	*10*	153	*182*	7	*15*	90	*55*	1515	*1893*
	3	13	46	50	*11*	137	*150*	3	*3*	67	*33*	784	*640*
	4	10	100	42	*11*	175	*244*	7	*9*	61	*32*	564	*365*
	5–6	11	64	46	*9*	412	*454*	7	*12*	52	*36*	1096	*991*
	7–8	9	67	55	*13*	496	*537*	6	*4*	62	*38*	705	*343*
D	1–5	16	69	61	*11*			49	*24*	247	*184*	14600	*16000*
	6–7	15	33	67	*11*			58	*33*	108	*59*	6200	*3600*
	8–10	12	58	77	*8*			56	*35*	101	*65*	5700	*4900*
E	2–3	20	45	43	*9*			6	*4*	103	*46*	812	*1370*
	4–5	16	75	49	*11*			13	*21*	83	*41*	1540	*2670*
	6–7	41	61	61	*9*			15	*19*	77	*55*	2040	*3700*
	8	28	68	63	*7*			23	*30*	58	*33*	1400	*1600*
	9–10	30	50	66	*12*			28	*25*	66	*42*	1860	*3700*

fluid (Figure 1, Table 2). The average concentration in the two trauma groups, as well as in the patients with acute pyrophosphate arthritis or with arthrosis only, was elevated (Table 2). Likewise, the total amount of proteoglycan fragments in the joint fluid, calculated as the sum of the mass of proteoglycan in the initially aspirated fluid and that in the saline washout, was higher in all the groups than in the reference group (Figure 2, Table 2). In addition, the average concentration of joint fluid proteoglycan in patients with pyrophosphate arthritis was higher than that in patients with arthrosis only.

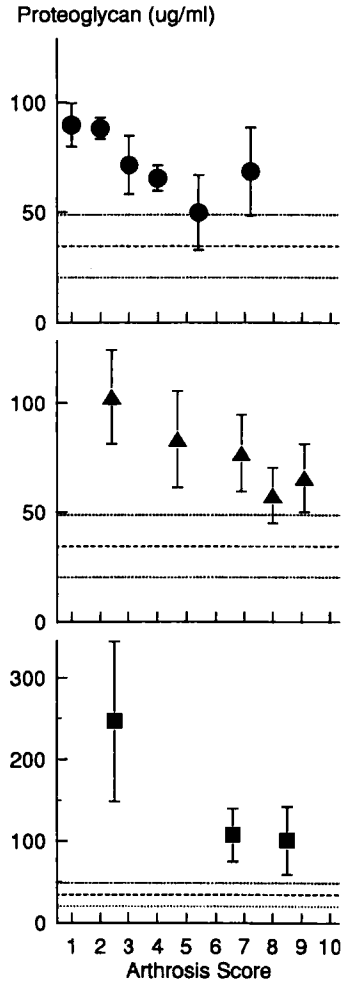

Figure 3. The concentration of proteoglycan fragments in joint fluid (µg/mL) related to the arthrosis score. At the top the combined results of the two trauma groups (B, C; ●). In the middle patients with arthrosis only (E; ▲). At the bottom, patients with pyrophosphate arthritis and arthrosis (D; ■). Patients with pyrophosphate arthritis and no radiological signs of arthrosis (score <6) and who were not examined by arthroscopy were assigned a "dummy value" of 2 for the arthrosis score for the purpose of the graph. Bars represent 95 percent confidence interval of the mean. The average for the healthy athletes is indicated by (------), with the 95 percent confidence interval indicated by (·····).

Concentration of proteoglycan fragments in relation to arthrosis score

When the patients were classified according to their arthrosis score, an inverse relation between the score and the average concentration of joint fluid proteoglycan was evident in all the groups (Figure 3). There was no difference between the average concentration of joint fluid proteoglycan fragments in patients with the same score from the two different trauma groups (Table 2). The concentration of proteoglycan fragments in injured joints with a greater degree of cartilage destruction approached that of the reference group. The group with the most advanced radiographic changes again showed increased average values, due to high concentrations in a few patients. 20 patients with a cruciate ligament injury and no meniscal tear, included in Group B, had results in accordance with the rest of the trauma patients. The average concentrations in the pyrophosphate groups were higher than in the reference group at all score levels, whereas all the patients with primary arthrosis, except those with an arthrosis score of 8, had elevated concentrations (Table 2).

Concentration of proteoglycan epitope in relation to age or time since injury

As was to be expected, increasing age was strongly related to an increasing arthrosis score. However, when the independent influence of age or time after injury on joint fluid proteoglycan concentration at each discrete arthrosis score level was analyzed, we found no relation between these two variables in either of the groups. There was no independent influence of sex on the joint fluid marker concentration.

Proteoglycan fragments in relation to joint fluid cell count

There was no relation of the synovial fluid cell count with the joint fluid proteoglycan fragment levels in patients with pyrophosphate arthritis (Figure 4).

Total mass of joint cartilage

The average cartilage mass (wet weight) in four healthy knees was 9.7 g (6.2–12.7), while the average wet weight of the menisci was 5.8 g in each knee. The total content of cartilage proteoglycan per joint was 630 mg (512–795) or 6.5 percent of the wet weight, while the average proteoglycan content in the menisci was 25 mg. At the time of joint replacement, the five knees with arthrosis (score 8–9) contained an average of 5.8 g (3.5–10.4) of cartilage with an average of 340 mg (121–746) of proteoglycan each or 5. 8 percent of the wet weight.

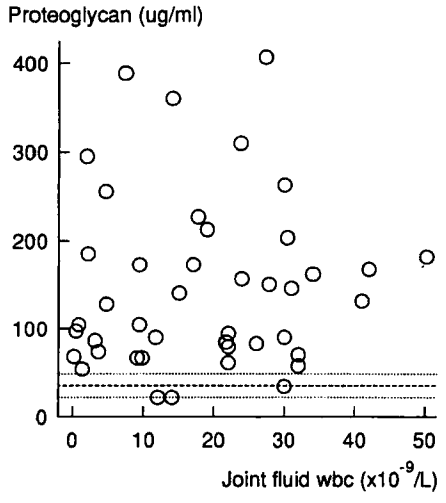

Figure 4. Proteoglycan fragment concentration in joint fluid in relation to joint fluid cell count in pyrophosphate arthritis.

Discussion

The need of a preradiographic diagnosis of joint cartilage disease has stimulated efforts to develop molecular markers for the detection of early stages of arthrosis (Thonar et al. 1985, Witter et al. 1987, Fife 1988, Ratcliffe et al. 1988, Sweet et al. 1988, Carroll 1989, Lohmander et al. 1989). A common finding in all of these investigations is, irrespective of the marker used, a considerable range of concentrations within the different groups of patients with arthrosis. We have, in the present study, attempted to identify causes of this variation. We find that the stage of development of the arthrosis, expressed as an arthrosis score, is an important cause of variation in joint fluid proteoglycan fragment concentration. Moreover, we demonstrated that at comparable arthrosis score levels, patients with acute pyrophosphate arthritis have higher levels of joint fluid proteoglycan than patients with arthrosis only. This strongly indicates an additional and independent influence of inflammatory activity on joint fluid markers. On the other hand, in these groups of patients, age, sex, and time after trauma did not seem to be potent independent factors influencing joint fluid proteoglycan concentration.

An inverse relation between joint fluid proteoglycan fragment concentration and radiographic disease progress has been observed in rheumatoid arthritis also (Saxne et al. 1985). In the present study, we have corroborated and extended this finding to both the early, arthroscopic stages and the later radiographic stages of arthrosis. We further demonstrate that a joint with an arthrosis score of 8 or 9 is accompanied by a lower cartilage and proteoglycan mass of about 50 percent, as compared with a normal joint. We thus propose that the cartilage content of the joint is one important factor which determines joint fluid cartilage marker concentration in arthrosis.

The concentration of cartilage degradation products in joint fluid is also influenced by variations in the rate of clearance of synovial fluid and its different components (Wallis et al. 1987, Levick 1990). Such variations are, however, difficult to determine in the individual patient and were not analyzed in the present study. It is likely, however, that in the arthrotic knee factors more directly related to the cartilage itself determine proteoglycan concentrations in the joint. The correlation between cartilage mass and proteoglycan concentration supports this contention.

Earlier, we have demonstrated that, after knee injury, synovial fluid proteoglycan fragment concentration is elevated over that of normal controls for many years, and we suggested that this increase is the result of an abnormal mechanical stress on the joint, possibly combined with a recurrent low grade synovitis (Lohmander et al. 1989). This may, in the early stages of the disease, elicit a repair response from the chondrocytes with a concomitant increase in both synthesis and degradation of cartilage matrix components (Adams 1989). Many of the posttraumatic joints demonstrate cartilage damage on arthroscopy, which may reflect early stages of arthrosis. With continued abnormal loading the attempts at repair by the chondrocytes are eventually overcome and the balance shifts in favor of degradation, resulting in a net loss of cartilage matrix from the joint. This loss of matrix, possibly combined with a decreased chondrocyte activity, may cause the decrease in the concentration of cartilage matrix components in synovial fluid, which we observe in the present study. This suggested sequence of events is supported both by observations on human arthrotic joint cartilage and by animal studies (Mankin et al. 1971, Vignon et al. 1983, Carney et al. 1984, Sandy et al. 1984, Adams 1989, Bulstra et al. 1989).

There was no obvious relationship between the cell count and the levels of proteoglycan in joint fluid in pyrophosphate arthritis. This may suggest that the inflammatory cells in synovial fluid do not play a primary role in the release of cartilage matrix components from the tissue in this condition. A lack of correlation between synovial fluid cell count and proteoglycans has also been noted in antigen-induced arthritis in rabbits and in rheumatoid arthritis (Pettipher et al. 1989, Bensouyad et al. 1990). In addition, joint destruction is not inhibited in arthritic animals genetically deficient in neutrophil elastase or cathepsin G

(Schalwijk et al. 1988). On the other hand, crystals in synovial fluid induce cytokine release from macrophages (Alwan et al. 1989) and animal studies have demonstrated that the degradative activity of chondrocytes is greatly stimulated by cytokines such as interleukin-1 or tumor necrosis factor (Saklatvala et al. 1985, Saklatvala 1986). The complexity of this issue is further illustrated by the inverse relation between interleukin-1 beta levels and proteoglycan in joint fluid demonstrated in rheumatoid arthritis (Saxne et al. 1988).

On the basis of our present and previous investigations, we conclude that synovial fluid concentrations of proteoglycan fragments in arthrosis are influenced by (1) the amount of cartilage matrix remaining in the joint, (2) the inflammatory activity and (3) the metabolic activity of the cartilage cells. These results emphasize the need for a detailed characterization of patients included in studies of markers of joint disease.

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