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Synovial fluid features and their relations to osteoarthritis severity: new findings from sequential studies

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Summary

Objective: Many factors are involved in the osteoarthritic process. It is not yet known which are initiators, promoters or simply results. Thus, we have evaluated some of those potentially important factors in osteoarthritis (OA) as observed sequentially for the first time in synovial fluids.

Design: Synovial fluids (SF) obtained between 1992–2002 were all routinely evaluated for gross appearance, leukocyte counts and microscopic examination of wet drop preparations. We used regular and polarized light and alizarin red s stains. We separated out all OA patients, then we looked for patients who had more than two synovial fluid analyses to get sequential information. Time between first and final aspiration ranged from 2 to 7 (3.6±1.6) years and number of analyses per patients from 3 to 6 (3.3±0.7). We related synovial fluid crystals, fibrils and white blood cell count (WBC) to age, sex, disease duration and radiographic assessment according to the Kellgren–Lawrence radiographic rating system.

Results: Of 4523 synovial fluid examinations, we found 855 in patients with knee OA; 330 patients with adequate clinical details for comparison were included in our study. Twenty-six patients (one woman and 25 men) had sequentially examined SF.

We found that 52% of those OA patients with effusions studied had crystals identified in their synovial fluid. Twenty-one percent of all the patients had CPPD crystals, 47% had hydroxyapatite, also called basic calcium phosphate (BCP) crystals and 16% had both types of crystals. Microscopically identifiable fibrils were found in 60% of SF.

In sequentially examined patients, CPPD crystals and apatite (BCP) were found in 19% and 23%, respectively, at the first aspiration and, in 34% and 58% at the final aspiration. Fibrils were seen in 54% at first examination and 85% later. Apatite and fibrils showed more significant correlation with time (r=0.51, r=0.92) than did CPPD (r=0.32). SF WBC correlated only with CPPD crystals and did not increase with OA duration or severity. CPPD, apatite and fibrils all correlated with higher radiographic grades of OA.

Conclusions: As noted before CPPD and apatite crystals were more common in patients with more severe OA. New findings are that our sequential cases showed that there were some patients with no crystals at onset but that crystals appeared with progression of the disease. Fibril presence in SF also correlated with progression of the disease. © 2003 OsteoArthritis Research Society International. Published by Elsevier Science Ltd. All rights reserved.

Key words: Osteoarthritis, Fibrils, Crystals, CPPD, Hydroxyapatite, Apatite, BCP.

Introduction

Osteoarthritis (OA) is a well-known disease since the Egyptian mummies, and is in part an aging process and one of the most common diseases[1]. However, much of our knowledge about its pathogenesis was formed in the last part of the twentieth century. There is now strong evidence that many factors ranging from the mechanical to biochemical and genetic join that process[1,2].

OA is characterized by degeneration of the cartilage with associated changes in bone. There is usually a mild degree of synovial inflammation, and calcium pyrophosphate or apatite crystals are often present[3–6]. The end pathological process in OA is an imbalance between joint cartilage synthesis and degradation resulting in cartilage loss. The crystals have a still undefined role in this process[7,8]. When one looks at SF in advanced OA, one or both crystals are seen in high percentages. These crystals were found in up to 60% of all patients with knee effusions in OA and 100% of small group of patients with grade-4 OA[4]. In destructive OA at a large joint such as the Milwaukee shoulder syndrome, calcium crystals are also seen in 100% of patients[9]. Calcium containing crystals may follow or precede destructive joint changes[7]. Crystals, whether primary or secondary to tissue degeneration, may accelerate the osteoarthritic process[10,11].

Fibrils, described in synovial fluid by Hollander in 1960[12], are a neglected microscopic finding. Using phase contrast microscopy, Kitridou *et al*. found fibrils in most synovial fluids and, showed that some of these fibrillar structures are indeed collagen fibers[13]. Presumably these are products of the core collagenous structure of cartilage[14,15]. One might expect a correlation between these fibrils and severity of OA but this has not been examined.

The objective of this study was to investigate the frequency and timing of appearance of calcium pyrophosphate dihydrate (CPPD) and apatite crystals and fibrils in

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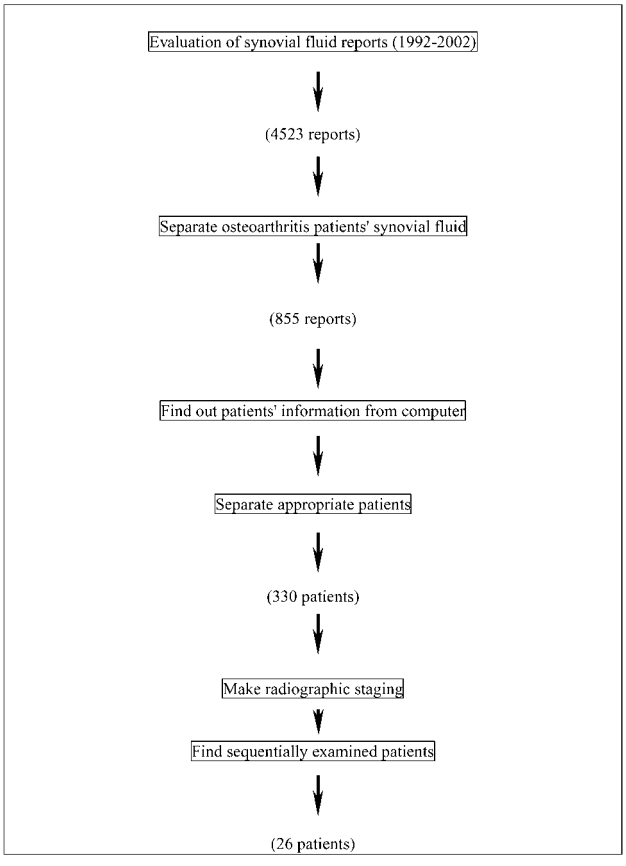

Fig. 1. Schema of study.

Table I
General characteristics of the patients

	Total (n=330)	Female (n=46)	Male (n=284)
Age (year)	65.22±11.85	61.02±13.81	65.9±11.39
X-ray grade (0–4)	2.63±1.19	2.17±1.25	2.7±1.17
SF WBC (×10^3/ml)	174.92±172.66	154.76±168.49	176.08±169.98
Fibrils	197 (60%)	21 (46%)	176 (62%)
CPPD	70 (21%)	12 (26%)	58 (20%)
Apatite	155 (47%)	17 (37%)	138 (49%)
Apatite+ CPPD	54 (16%)	10 (22%)	44 (15%)

synovial fluid in OA. Are the crystals in this process results, promoters or both?

PATIENTS AND MATERIALS

We evaluated all synovial fluid analysis reports on specimens obtained between 1992 and 2002 at the Philadelphia VA Medical Center and identified those from knee OA patients (Fig. 1), diagnosed according to American College of Rheumatology criteria based on both clinical and radiological evidence[16,17]. Patients with other rheumatic diseases that affect knees (particularly rheumatoid arthritis and gout) were excluded. We recorded synovial fluid results, age, sex and radiographic assessment from computerized

Table II
Statistical comparison of some variables among OA patients

	P values			
	X-ray grade	Fibrils	Apatite	CPPD crystals
Age (year)	0.0001	0.93	0.1	0.017
WBC (×10^3/ml)	0.13	0.83	0.5	0.01
Fibrils	0.0005	—	0.35	0.13
CPPD crystals	0.004	0.13	0.0001	—
Apatite	0.005	0.35	—	0.0001
X-ray grade (0–4)	—	0.0005	0.005	0.004

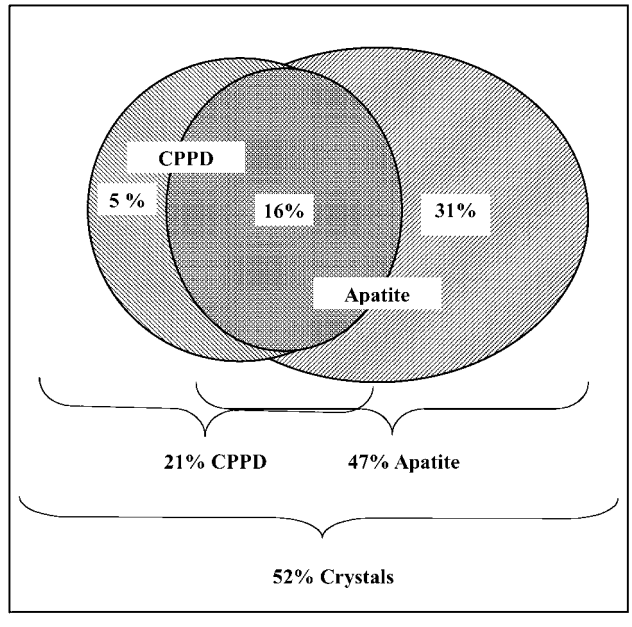

Fig. 2. Graphical documentation of results for the crystals.

records of patients' archives. Radiographic grading of knee films was by the Kellgren–Lawrence radiographic rating system[18].

Finally, we recorded patients whose SF had been re-examined more than two times to get sequential information about relations between the crystals, fibrils, WBC and the disease process. Time between first and final aspiration ranged from 2 to 7 (3.6±1.6) years and number of analyses from 3 to 6 years (3.3±0.7).

Synovial fluid (SF) analyses for crystals had been performed by examination of wet drop preparations of SF under regular and polarized light[19]. CPPD crystals were considered present when crystals of rhomboid shape with weak positive birefringence were observed. Apatite crystals were suspected by presence of glossy non-birefringent chunks staining positively with alizarin red s and recorded as 0–3+[19,20]. We accepted all with 1+ and more as positive. Fibrils were considered present when clumps were seen in at least two high power fields in wet drop preparations[13,19,21].

STATISTICAL ANALYSIS

All data are expressed as the mean±S.D. Paired samples were analysed using Mann–Whitney U or Wilcoxon-rank tests for matched pairs when they were needed. Linear

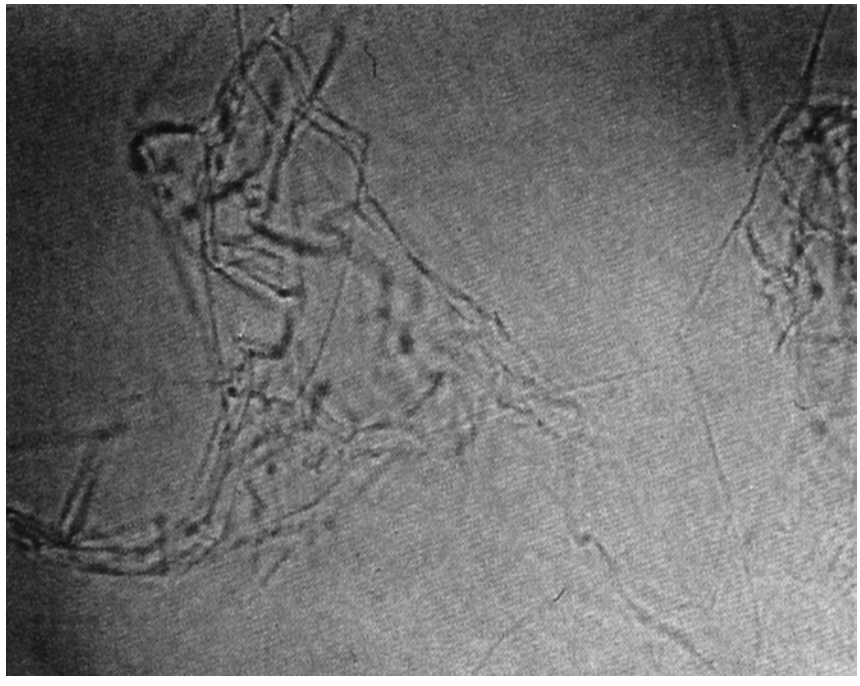

Fig. 3. Fibrils in synovial fluid (ordinary light, ×300) are long strands with a ragged and angulated appearance. This appearance distinguishes them from crystals.

Table III
Synovial fluid findings on sequential visits

	First visit	Last visit	*P*
CPPD	19% (5/26)	34% (9/26)	0.046
Apatite	23% (6/26)	58% (15/26)	0.005
Fibril	54% (14/26)	85% (22/26)	0.005

regression analysis was used for sequential patients to examine the relatonship of duration with fibrils, CPPD and apatite crystals. *P* values less than 0.05 were considered significant.

Results

As shown in Fig. 1, 855 of 4523 synovial fluid examination reports were on OA fluids. Of the 855 patients with knee osteoarthritis, 330 patients were included to our study because others had missing data or they also had some other rheumatologic diseases. Only 26 patients (one woman and 25 men) were accepted as sequentially examined patients (Fig. 1).

We found 52% (171/330) of the OA patients had crystals identified in their synovial fluid. Twenty-one percent of all the patients with crystals had CPPD crystals, 47% had apatites and 16% had both types of crystals (Table I, Fig. 2). Fibrils were found in 60% of patients (Fig. 3).

Table II summarizes the correlations among the variables examined. OA patients with older ages were associated with increased frequency of CPPD crystals and higher radiographic grades (P=0.0001). However, synovial fluid WBC was correlated only with CPPD crystals (P=0.01). Fibrils, CPPD crystals and apatite crystals were all correlated with higher radiographic grades of OA (P<0.05). CPPD and BCP were significantly encountered together (P=0.0001).

In sequentially examined patients, CPPD and apatites were present in 19% and 23% respectively at the first visit and in 34% and 58% at the last visit. Fibrils were seen in 54% at the beginning and in 85% later (Table III). The difference of frequencies between first and last visits for CPPD, apatite and fibrils were significant (P<0.05) (Table III). The frequencies of the development of CPPD, apatite and fibrils in knee joints of OA patients initially found negative for these variables were 15%, 35% and 31% respectively. Apatite crystals and fibrils showed more significant correlation with duration in sequential examinations (r=0.51, r=0.92) than CPPD did (r=0.32).

Discussion

Compared to the literature, our percentage of SF with crystals in OA (52%) was in the middle of the reported range of 41% to 60%[4,5]. Some of the variation in that range may be due to different techniques used. Our study also gives very similar results to the previously reported results on the relation of calcium containing crystals to OA severity[22–24]. The correlation between crystal frequency and higher radiologic grade of OA would be consistent either with the thesis that the crystals contribute to the severity, or that crystal presence results from more severe disease[4].

Our important finding is the first documentation that OA patients who did not have CPPD or apatite crystals on the first synovial fluid studied had these crystals detected later. This strongly supports that some crystals are a result of the OA process. In one previous retrospective sequential study, Reuge and co-workers[25] identified that 25% of 44 OA patients who initially had no radiographic evidence of joint calcification developed either X-ray evidence of calcification or CPPD crystals in SF over a mean of 8 years. Not

all had SF examinations and apatites (BCP) were not considered. We found that about 15% of our cases with SF without CPPD later developed the crystals while 31% developed apatites.

It is known that the prevalence of CPPD crystal deposition in the knee increases with age[26–28], CPPD presence also correlated with age in our study. In contrast, apatite presence correlated only with OA severity by X-ray.

A striking finding in our study was the very significant correlation of fibrils with radiologic stage of OA ($P=0.005$). Fibrils as defined in our analyses were found in 60% of OA fluids and showed correlation with duration of the disease but not with age ($P=0.93$). Thus SF fibrils may be an independent consequence of some pathologic events in the OA process.

Birefringent fibril strands that are morphologically indistinguishable from sloughed collagen fibers may be visualized on wet mount microscopy (Fig. 3)[19]. It is impossible, however, to be certain that all fibrils seen in a wet smear of synovial fluid under phase contrast or polarized light microscopy are indeed collagen. Fresh fibrin, for instance, may not be easily distinguished from collagen fibrils[12]. Swann noted collagen fibers as an incidental finding during an electron microscopic study of SF[29]. On the other hand, Cheung *et al.* using gel electrophoresis and spectrophotometry also showed that the presence of collagen (about 5 μg or more) was correlated with roentgenographic evidence of joint space narrowing in OA and other arthropathies[30]. Fibrils in our study correlated highly with radiographic OA but not with CPPD or apatites. More study is needed to characterize fibrils and collagen in SF as possible factors in pathogenesis. Other techniques to quantify collagen in SF will be of interest[31].

It is generally agreed that there is evidence for the presence of a mild synovitis in OA joints[32–34]. In our study inflammation was evaluated with SF WBC, which had a correlation only with CPPD presence. Our SF WBC did not correlate with apatite presence. SF WBC probably does not reflect all synovial tissue changes.

Male dominance in our VA hospital population may limit for generalizability of the results. However, we know of no studies suggesting different patterns of crystal deposition in males vs females.

This study has confirmed that fibrils, CPPD and apatite crystals are found in SF in the osteoarthritic process. Also, we sequentially showed that there were some patients with no crystals and no fibrils at onset but they appeared with progression of the disease. The role(s) of these materials in synovial fluid invite further study.

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