The High Prevalence of Pathologic Calcium Crystals in Pre-operative Knees

BETH A. DERFUS, JASON B. KURIAN, JEFFREY J. BUTLER, LAUREEN J. DAFT, GUILLERMO F. CARRERA, LAWRENCE M. RYAN, and ANN K. ROSENTHAL

ABSTRACT. ***Objective.*** Calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals are important in the pathogenesis of osteoarthritis (OA) but are under recognized even in end stage disease. We determined the prevalence of these calcium crystals in synovial fluid (SF) of persons undergoing total knee arthroplasty for degenerative arthritis.

Methods. SF samples were obtained from 53 knee joints undergoing total arthroplasty for a pre-operative diagnosis of OA. SF were analyzed via compensated light microscopy for CPPD crystals and a semiquantitative radiometric assay for BCP crystals. Fifty pre-operative radiographs were analyzed and graded according to the scale of Kellgren and Lawrence.

Results. Patients had an average age of 70 years at the time of surgery. CPPD and/or BCP crystals were identified in 60% of SF. Overall radiographic scores correlated with mean concentrations of BCP crystals. Higher mean radiographic scores correlated with the presence of calcium-containing crystals of either type in SF. Radiographic chondrocalcinosis was identified in only 31% of those with SF CPPD.

Conclusions: Pathologic calcium crystals were present in a majority of SF at the time of total knee arthroplasty. Intraoperative SF analysis could conveniently identify pathologic calcium crystals providing information that may be relevant to the future care of the patient's replaced joint and that of other joints. This information could also potentially aid in predicting the likelihood of the need for contralateral total knee arthroplasty. (J Rheumatol 2002;29:570–4)

Key Indexing Terms:
CALCIUM PYROPHOSPHATE PSEUDOGOUT OSTEOARTHRITIS
ARTHROPLASTY CHONDROCALCINOSIS BASIC CALCIUM PHOSPHATE

Osteoarthritis (OA) is the most common indication for total joint replacement. Calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals including apatite are active participants in the pathogenesis of OA[1]. Intraarticular calcium crystals may provoke acute attacks of painful synovitis [2,3]. More often they are associated with degenerative arthritis that is more advanced than in patients with OA but no intraarticular crystals. Specifically, more severe radiographic degenerative changes[4] and larger joint effusions[5] are observed in patients whose degenerated joints contain calcium crystals. In animal models, OA worsened after introduction of CPPD crystals[6]. *In vitro* studies suggest that calcium crystals likely play a role in causing or amplifying degeneration of cartilage. CPPD and BCP crystals increase *in vitro* chondrocyte mitogenesis, metalloproteinase elaboration, and prostaglandin E_2 production[7,8]. Calcium crystals may also be markers for accelerated cartilage degeneration. Synovial fluid (SF) studies indicate more proteases and cartilage breakdown products in calcium crystal-related OA than in idiopathic form[9].

Articular calcium crystals are under recognized, unfortunately even in end stage OA. Radiographic chondrocalcinosis is seen in a minority of persons with articular CPPD or BCP crystals[10]. Radiographic studies are particularly insensitive in advanced disease where the cartilage is attenuated. Identification of calcium crystals in SF requires expertise in the use of compensated polarized light microscopy to detect CPPD and a unique semiquantitative radiometric assay to detect BCP[4]. Diagnostic SF crystal analyses are, therefore, not routinely performed even when such fluid is abundantly available at the time of total joint arthroplasty.

Health care costs for OA approach an estimated 1–2.5% of the gross national product[11]. Over 500,000 persons with

From the Departments of Medicine and Radiology, Medical College of Wisconsin, the Department of Orthopedic Surgery, St. Luke's Medical Center, and the Zablocki VAMC, Milwaukee, Wisconsin, USA.

Supported by NIH grants AR-RO1-38656-10 (LMR), AG-RO1-15337 (AKR), a grant from the Schoenleber Foundation (BAD), and a VA Merit Review Award (AKR).

B.A. Derfus, MD, Assistant Professor of Medicine; J.B. Kurian, BS, Medical Student, Medical College of Wisconsin; J.J. Butler, MD, Staff Physician, Orthopedic Surgery, St Luke's Medical Center, Milwaukee, WI; G.F. Carrera, MD, Professor of Radiology, Chief, Diagnostic Radiology; L.M. Ryan, MD, Will and Cava Ross Professor of Medicine, Chief of Rheumatology, Medical College of Wisconsin; A.K. Rosenthal, MD, Associate Professor of Medicine, Medical College of Wisconsin, and Chief, Rheumatology Section, Zablocki VAMC.

Address reprint requests to Dr. A.K. Rosenthal, Rheumatology Section, cc-111W, Zablocki VAMC, 5000 W. National Ave., Milwaukee, WI 53295-1000. E-mail: Ann.Rosenthal@med.va.gov

Submitted April 16, 2001; revision accepted August 16, 2001.

OA undergo surgical procedures each year. The estimated cost per hospitalized OA patient approaches 50% of the median family income in the United States[11]. Total joint replacements raise the annual cost of care per patient with OA. Since calcium crystals are under recognized clinically despite ample evidence of their importance in the pathogenesis of OA, we sought to confirm the presence of these crystals at a physically and fiscally critical stage in the disease - the time of total joint arthroplasty. The identification of calcium crystals may have important implications for post-operative care as well as for our understanding of the pathogenesis of end stage OA.

MATERIALS AND METHODS

Synovial fluid. Between April 1998 and August 1998 SF samples were obtained at the time of 53 randomly selected primary total knee arthroplasties performed for degenerative arthritis as indicated by the operating surgeon. Surgeries were performed at 4 metropolitan hospitals by 14 orthopedic surgeons. The average age of persons undergoing total knee arthroplasty was 70 years (range 52–90). The average age of the 21 men in the study was 71 years (range 61–80). The average age of the 32 women in the study was 70 years (range 52–91).

Radiographs. Radiographs were obtained by the method standard for the practice of the attending surgeons. Preoperative radiographs were read blindly by a skeletal radiologist (GFC) and graded using the scale of Kellgren and Lawrence in which 0 = normal; 1 = doubtful joint space narrowing and possible osteophyte; 2 = mild; definite osteophyte or possible narrowing; 3 = moderate; moderate/multiple osteophyte(s) or definite narrowing, and 4 = severe; large osteophyte or marked narrowing[12].

Synovial fluid cell counts. SF specimens were aspirated immediately prior to the onset of total joint arthroplasty. Fluids were stored in sterile sodium heparin-containing vacutainers (Becton Dickinson) for 2-4 hours at 4°C, and analyzed for cell count and crystals on the day of surgery. White blood cell counts were performed by diluting synovial fluids in 2% crystal violet in 0.9% sodium chloride, then counting in an Improved Neubauer hemocytometer (Reichert, Buffalo, NY, USA).

Compensated polarized light microscopy. SF were analyzed for CPPD using a Leitz compensated polarized light microscope (Midland, Ontario, Canada). CPPD appeared as weakly, positively birefringent or non-birefringent rod- or rhomboid- shaped crystals. Visualization of at least 5 such crystals was necessary to confirm the presence of CPPD crystals.

^{14}C EHDP binding. The ^{14}C EHDP (ethane-1-hydroxy 1,-1-diphosphonate) binding assay is a semiquantitative assay to detect BCP crystals. EHDP is a bisphosphonate that binds to the surface of BCP crystals. Because the surface area/mass ratio may vary for different sized crystals, it can only be used to roughly estimate the quantity of BCP crystals present.

Three milliliters of SF was incubated at 27° C for 30 min with 250 units of sodium heparin (Elkins-Sinn, Inc., Cherry Hill, NJ, USA) and 2000 USP units of hyaluronidase (Worthington Biochemical, Lakewood, N.J.) (4). BCP crystals were then pelletted via ultracentrifugation at 27,000g for 20 minutes in a Sorvall RC2-B. Pelletted crystals were suspended in 0.1M Tris, pH 8.0 to a total volume of 1 ml. This solution was incubated with 100 μl of 1% trypsin (weight/volume) (Worthington) for 30 minutes at 37°C. Calcium crystals were again pelletted via ultracentrifugation at 36,000 × g for 20 minutes. This pellet was suspended in 1 ml of 0.2 M phosphate buffered saline, pH 7.2. One milliliter of a mixture containing 340 nanocuries ^{14}C EHDP (Norwich-Eaton Pharmaceutical, Norwich, NY, USA) in phosphate buffered saline was added to the suspended pellet and rotated end-over-end at 4°C for 3 hours.

One hundred microliter samples of this mixture were added to 5 ml Scintisafe solution (Fisher, Pittsburgh, PA, USA). The remaining specimen was ultracentrifuged at 36,000 × g for 20 min and 100 ml samples of the supernatant were similarly added to Scintasafe solution. All were counted in a Minaxi Tri-Carb 4000 series scintillation counter (Packard, Downers Grove, IL, USA). Standard samples containing 10, 25, 50, and 100 μg of sonicated hydroxyapatite were similarly analyzed as quality controls.

The total counts in the ultracentrifuged specimens were subtracted from the total counts in the uncentrifuged specimens to obtain the total number of counts in the pellet. Percentage of ^{14}C-binding (EHDP binding) was determined by dividing the difference between the total number of counts in the pellet by the total number of counts in the uncentrifuged specimens and multiplying by 100. Duplicate determinations were made on each sample. Normal SF shows less than 8% ^{14}C EHDP binding, which corresponds to about 10 μg/ml apatite standard.

Statistics. Differences between proportions were analyzed via chi square analysis. The Wilcoxon rank sum test was used to compare groups of data.

RESULTS

All SF cell counts were either in the normal or non-inflammatory range. Twenty-one SF samples were normal containing < 200 white blood cells (WBC)/mm^3. Twenty samples were non-inflammatory containing 200–2000 WBC/mm^3 (Table 1). The presence of SF calcium crystals could not be predicted by the gross appearance of the fluids or by the intraoperative appearance of the knee joint.

CPPD and/or BCP crystals were identified in 60% (32 of 53) of SF at the time of total knee replacement (Table 2). CPPD crystals were identified in 30% of fluids examined, while BCP crystals were identified in 49%. Both crystal types were identified in 19% of SF samples. CPPD was found more often in women's SF than in men's (Table 3) [41% (13/32) vs 14% (3/21)]. In patients over age 70 years, 85% showed SF calcium crystals compared to 37% for those

Table 1. White blood cell (WBC) counts in synovial fluid with and without calcium crystals.

Crystal Type (n)	WBC mm^3 Normal	Noninflammatory	Not Done
CPPD (6)	3	2	1
BCP (16)	6	6	4
Both (10)	3	5	2
None (21)	9	7	5

Table 2. Types and rates of calcium crystals in pre-operative synovial fluid.

Crystal Type	Prevalence % (fraction)
CPPD alone	11 (6/53)
BCP alone	30 (16/53)
CPPD and BCP	19 (10/53)
CPPD or BCP	60 (32/53)
No crystals	40 (21/53)

Table 3. Gender-specific prevalence of synovial fluid calcium crystals.

Crystal Type	Prevalence of Synovial Fluid Calcium Crystals	
	Women, n = 32 % (fraction)	Men, n = 21 % (fraction)
CPPD alone	19 (6/32)	0 (0/21)
BCP alone	22 (7/32)	43 (9/21)
CPPD and BCP	22 (7/32)	14 (3/21)
CPPD or BCP	63 (20/32)	57 (12/21)
No crystals	37 (12/32)	43 (9/21)

70 years and younger. Although there was considerable overlap between the ages of persons with and without SF crystals, the group with crystals proved significantly older than that without ($p < 0.0003$).

In no case was calcium crystal-related arthropathy listed as a pre-operative diagnosis (Table 4), although a majority of SF contained these crystals. Chondrocalcinosis was present on pre-operative radiographs in only 31% (5/16) of those with SF CPPD crystals. In one patient's SF sample previously unsuspected monosodium urate crystals were identified.

Of fifteen joints with severe, grade 4 radiographic degeneration, 93% (14/15) contained intraarticular calcium crystals (Table 5). Joints containing calcium crystals had a higher mean radiographic score than those without crystals (3.45 *vs* 2.95). Variability of available radiographic views may have limited radiographic scores in some cases. Higher mean concentrations of BCP crystals were also associated with more severe radiographic degeneration (Figure 1). The simultaneous presence of both types of calcium crystals was associated with the highest mean levels of BCP binding.

DISCUSSION

Table 4. Pre-operative and post-operative diagnoses in patients undergoing knee arthroplasty.

Disease	Pre-operative Diagnosis	Post-operative Diagnosis
OA no crystal	53	21
OA with CPPD	0	16
OA with BCP	0	26
Gout	0	1

Table 5. Radiographic scores in patients with and without synovial fluid calcium crystals.

Crystal Type	Radiographic Score (% prevalence)				
	1	2	3	4	5
No Crystals (n = 21)	0	0	11	84	5
CPPD or BCP (n = 32)	0	0	0	55	45

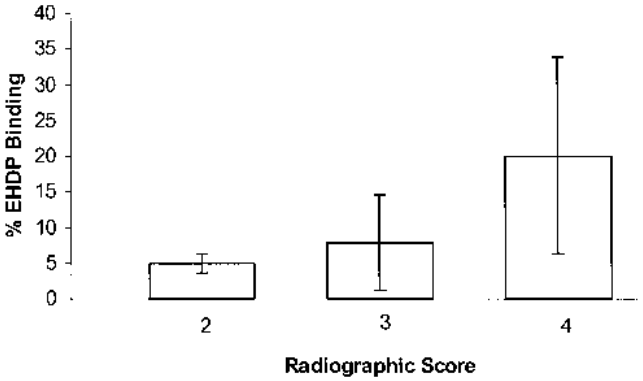

Figure 1. Correlation of radiographic score with mean percentage of EHDP binding. EHDP = ^{14}C ethane-1-hydroxy 1,-1-diphosphonate.

We observed that 60% of patients undergoing total knee arthroplasty have pathologic calcium crystals in their SF. These findings suggest that in these clinically end-stage joints, both CPPD and BCP crystals are far more common than once believed. There is ample evidence that these crystals are not simply innocent bystanders, but active participants in cartilage degeneration[10]. Both BCP and CPPD crystals increase mitogenesis and protease release from articular cells[7,8]. CPPD crystals also accelerate joint degeneration in animal models of OA[6] and may contribute mechanically to cartilage matrix destruction[13]. We believe that the high prevalence of BCP and CPPD crystals is important to our understanding of the pathogenesis of OA, and may ultimately influence the clinical care of these patients.

Several previous studies examined the prevalence of pathologic calcium crystals in SF from patients with OA. In another perioperative study, 52% of patients over the age of 75 had cartilage CPPD crystals at the time of total joint replacement[14]. In typical OA SF obtained from non-perioperative patients, 60-71% contained calcium crystals[15,16]. However, 83% of a subset of patients with rapidly progressive OA had calcium crystals[9]. When articular tissues were included in the analysis, 80% of typical OA and 100% of rapidly progressive OA contained calcium crystals. Interestingly, our results suggest that the percentage of patients with calcium crystals in their fluid may be similar in a non-perioperative population with OA. Our similar rates may reflect the severity of the disease balanced by more accurate methods to detect BCP crystals (less false positives). We found the most common type of crystal in pre-operative fluids was BCP alone. These data vary somewhat from previous data showing that the combination of BCP and CPPD crystals was more common than either crystal alone[15,16]. Because the presence of BCP crystals correlates with the severity of radiographic degeneration in the joint, one may expect a higher prevalence of this crystal type in the current cohort selected for severe disease.

We show here that calcium crystals are more common in

patients over the age of 70 and that CPPD crystals are more common in women than in men. This mirrors epidemiologic data showing a dramatic increase in chondrocalcinosis with age, with the prevalence doubling during each decade over the age of 60[17]. Symptomatic CPPD deposition disease may be more common in women than men[3]. Similarly clinical arthritis associated with BCP crystals, such as Milwaukee shoulder syndrome, is rare in patients under age 60[18].

Our radiographic findings correlate with the previously described association between BCP crystals and radiographic signs of joint degeneration[4,15]. However, only a handful of our patients with CPPD crystals in their SF had radiographic chondrocalcinosis. This may be attributed to the variable quality of office radiographs, and the paucity of cartilage in end stage disease. Clearly, pre-operative radiographs are not sufficient to identify those patients with CPPD crystals in their SF.

Identification of calcium crystals in intraoperative SF specimens is clinically relevant for a number of reasons. First, the deposition of BCP and/or CPPD crystals is seldom monoarticular and may affect other large joints and slow postoperative recovery[3,19]. Second, the clinician needs to be aware of the potential for postoperative pseudogout attacks in those persons with CPPD crystals in their operative joint fluids[20,21]. Even the operated joint can be affected by pseudogout from CPPD crystals shed from residual cartilage or areas of synovial chondroid metaplasia[22]. Lastly, the primary care provider should investigate patients under age 60 with articular CPPD crystals for metabolic causes of CPPD deposition, such as hyperparathryoidism and hemochromatosis. We therefore suggest that crystal analysis be performed on fluids from all operated knee, hip, or shoulder joints.

Our study is not without limitations. These patients all had end stage disease and these findings certainly do not reflect the early stages of OA, where non-surgical interventions may prevent joint damage. Although calcium crystals are present in high percentages of patients coming to joint arthroplasty, we certainly cannot attribute all end stage joint disease to these crystals. Sixty-three percent of patients aged 70 and under had no crystals in their perioperative SF. Perhaps younger patients have other factors contributing to their joint disease, such as trauma or congenital abnormalities. Our study used a relatively small number of patients and they came from a single regional area. Thus, socioeconomic or regional influences may have swayed our results. Moreover, we used existing radiographs not specifically performed for the study. The techniques used for filming may have limited our ability to detect signs of crystals on radiographs. This, however, reflects true clinical practice.

In summary, we demonstrated a 60% prevalence of pathologic calcium crystals in SF of 53 patients undergoing joint replacement. These findings have important implications for clinicians and warrant further studies of clinical outcomes in patients undergoing joint replacement with and without pathologic crystals. We recommend routine crystal analysis on intraoperative SF from all patients undergoing arthroplasty of those joints potentially affected by crystal deposition, especially the knees.

REFERENCES

1. Cheung HS, Ryan LM. Role of crystal deposition in matrix degradation. In: Woessner FJ, Howell DS, editors. Joint cartilage degradation. Basic and clinical aspects. New York: Marcel Dekker; 1993:209-23.
2. Halverson PB, McCarty DJ. Basic calcium phosphate (apatite, octacalcium phosphate, tricalcium phosphate) crystal deposition diseases; calcinosis. In: Arthritis and allied conditions. Philadelphia: Lea & Febiger; 1997:2127-46.
3. Ryan LM, McCarty DJ. Calcium pyrophospahte dihydrate deposition disease, pseudogout, and articular chondrocalcinosis. In: Koopman WJ, editor. Arthritis and allied conditions. Philadelphia: Lea & Febiger; 1997:2103-25.
4. Halverson PB, McCarty DJ. Identification of hydroxyapatite crystals in synovial fluid. Arthritis Rheum 1979;22:389-95.
5. Carroll GJ, Stuart RA, Armstrong JA, Breidahl PD, Laing BA. Hydroxyapatite crystals are a frequent finding in osteoarthritic synovial fluid, but are not related to increased concentrations of keratan sulfate or interleukin 1ß. J Rheumatol 1991;18:861-6.
6. Fam AG, Morava-Protzner I, Purcell C, Young BD, Bunting PS, Lewis AL. Acceleration of experimental lapine osteoarthrits by calcium pyrophosphate microcrystalline synovitis. Arthritis Rheum 1995;83:201-10.
7. Cheung HS, Halverson PB, McCarty DJ. Phagocytosis of hydroxyapatite or calcium pyrophosphate dihydrate crystals by rabbit articular chondrocytes stimulates release of collagenase, neutral protease, and prostaglandins E2 and F2 alpha. Proc Soc Exp Biol Med 1983;173:181-9.
8. Cheung HS, Halverson PB, McCarty DJ. Release of collagenase, neutral protease, and prostaglandins from cultured mammalian synovial cells by hydroxyapatite and calcium pyrophosphate dihydrate crystals. Arthritis Rheum 1981;24:1338-44.
9. Bardin T, Bucki B, Lansman J, Lequesne M, Dryll A, Kuntz D. Calcium crystals and rapidly destructive osteoarthritis. Osteoarthritis Cartilage 1993;1:15.
10. Ryan LM, Cheung HS. The role of crystals in osteoarthritis. Rheum Dis Clin North Am 1999; 25:257-67.
11. Yelin EH. The economics of osteoarthritis. In: Brandt KD, Doherty M, Lohmander LS, editors. Osteoarthritis. New York: Oxford Press; 1998:23-30.
12. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16:494-502.
13. Hayes A, Harris B, Dieppe PA, Clift SE. Wear of cartilage:The effect of crystals. Proc Inst Mech Eng 1993;207:41-58.
14. Sokoloff L, Varma A. Chondrocalcinosis in surgically resected joints. Arthritis Rheum 1988;31:750-6.
15. Gibilisco P, Schumacher HR, Hollander JL, Soper KA. Synovial fluid crystals in osteoarthritis. Arthritis Rheum 1985;28:511-5.
16. Halverson PB, McCarty DJ. Patterns of radiographic abnormalities associated with basic calcium phosphate and calcium pyrophospahte dihydrate crystal deposition. Ann Rheum Dis 1986;45:603-5.
17. Felson DT, Naimark A, Anderson J, Kazis L, Castelli W, Meenan RF. The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. Arthritis Rheum 1987;30:914-8.
18. Halverson PB, Garancis J, McCarty DJ. Histopathological and ultrastructutural studies of synovium in Milwaukee shoulder syndrome - a basic calcium phosphate crystal arthropathy. Ann

Rheum Dis 1984;43:734-41.
19. Halverson PB, McCarty DJ, Cheung HS, Ryan LM. Milwaukee shoulder syndrome: Eleven additional cases with involvement of the knee in seven (basic calcium phosphate crystal deposition disease). Semin Arthritis Rheum;1984;14:34-44.
20. Masuda I, Ishikawa K. Clinical features of pseudogout attack. Clin Orthopaedics 1988;229:173-81.
21. O'Duffy D. Clinical studies of acute pseudogout attacks. Arthritis Rheum 1976;19:349-52.
22. Ishikawa K, Masuda I, Ohira T, Kumamoto S, Yokoyama M. A histological study of calcium pyrophosphate dihydrate crystal-deposition disease. J Bone Jt Surg 1989;71A:875-86.