The two faces of the same medal… or maybe not? Comparing osteoarthritis and calcium pyrophosphate deposition disease: a laboratory and ultrasonographic study

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Abstract

Objective

Osteoarthritis (OA) and calcium pyrophosphate deposition disease (CPPD) are frequently associated but the real relation between these diseases is not still understood. The aim of this paper is to investigate the characteristics in terms of inflammation, anatomical changes and synovial fluid (SF) features in knees of patients with OA and CPPD.

Methods

Consecutive patients older than 55 years with knee pain and swelling were enrolled. All patients underwent a complete clinical examination, a US examination of the affected joint, arthrocentesis of the knee and analysis of synovial fluid, including dosing of inorganic ions and number of crystals. The gold standard for the diagnosis was the microscopic analysis of the SF.

Results

Sixty-seven patients were enrolled, 25 affected by OA and 42 by CPPD. At US, a significantly higher amount of effusion and synovitis was identified in patients with CPPD but there were no significant differences regarding structural changes. At the SF analysis, the white blood cell (WBC) count was higher in patients with CPPD who also presented a higher number of polymorphonuclear cells and a lower number of monocytes. Regarding the inorganic ion concentration, the statistical analysis did not reveal any differences. The number of crystals in the SF, correlated with a larger effusion, higher grade of synovitis and a higher WBC count.

Conclusion

A higher degree of inflammation was found in patients with CPPD. The findings suggest that longitudinal studies would be useful to better understand the evolution of the diseases and highlight the need for different treatment strategies.

Key words

crystal arthropathies, osteoarthritis, ultrasonography, knee, synovial fluid analysis, calcium pyrophosphate deposition disease

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Introduction

Calcium pyrophosphate deposition disease (CPPD) is an umbrella term conceived by the EULAR task force in 2011 to describe all instances of occurrence of calcium pyrophosphate crystals (CPP) in joints (1). According to the taskforce definitions, CPPD may occur clinically with 4 different presentations. Two of them are easier to identify as they present typical signs of inflammation and are the acute attack of synovitis previously called pseudogout and the chronic inflammatory arthritis associated with CPPD. Regarding the other two forms however, namely the asymptomatic CPPD that can be also associated with osteoarthritis (OA) and the OA with CPPD, the role and the impact of CPPD in joint damage and symptoms is not clear, and OA is considered generally as the main responsible for the clinical features (1).

Only few papers tried to assess the relationship between OA and CPPD in the past, using however heterogeneous approaches. Conventional radiology studies have suggested that CPPD may present different sites of involvement if compared to OA alone, including the lateral femoral-tibialcompartment, the II and III metacarpal-phalangeal joints and the wrist joints (scapho-trapezial joint and lunate collapse) (2, 3) while it is not clear if it associates with distinct radiographic phenotypes of OA (4) concerning osteophytes and bone attrition. Regarding inflammation, a pilot study analysing synovial membranes of patients with CPPD and OA or OA alone, highlighted a different morphology of the samples suggesting different pathogenetic pathways for the two diseases (5). Basic research studies that focused on synovial fluids of patients with OA and OA plus CPPD demonstrated an increased concentration of transforming growth factor beta (TGF-β) and matrix metalloproteinasis 1 (MMP-1) (6) in patients without acute inflammation and higher titres of IL-6 in patients with acute attack (7). Regarding the concentration of inorganic ions in the synovial fluid, researchers focused mainly on inorganic pyrophosphate (PPi) demonstrating a higher concentration in patients affected by OA plus CPPD than OA alone (8, 9). In addition, dysregulated PPi and inorganic phosphate (Pi) metabolism, and changes in extracellular Ca^{2+} and Mg^{2+} levels have been suggested as determinants of CPP crystal formation (10, 11)

Several papers have been also published in recent years regarding imaging in CPPD and especially the applications of ultrasonography (US) in the diagnosis and assessment of CPPD. US has demonstrated to be an accurate (12, 13) and reliable (14, 15) technique in identifying CPP deposition in knees and wrists. Furthermore, a US study evaluated the extent and distribution of CPP deposition in joints, demonstrating that knee is the most frequently involved site (16).

Despite the apparently very high frequency of CPPD (17), many aspects of CPPD are far from being fully understood (18), especially the relationship with OA, the biochemical mechanisms that bring to the formation of CPP crystals and the pathogenetic pathways that provoke joint damage and inflammation in the two diseases and have been prioritised in the Gout, Hyperuricemia and Crystal Associated Disease Network (G-can) Research agenda for CPPD (19). The aim of this paper was to assess the differences, if any, in terms of inflammation and anatomical changes as assessed by ultrasound (US) and synovial fluid characteristics, including dosing of inorganic ions, in the knees of patients with OA with CPPD and OA alone.

Patients and methods

Consecutive patients over 55 years of age who went to the outpatients clinic of the Rheumatology Institute of the University of Siena from January 2018 to April 2018 for knee pain and swelling were enrolled in the study. Patients with known diagnosis of any chronic inflammatory arthritis were excluded. All patients underwent at the time of enrolment a complete clinical examination in order to rule out a polyarticular inflammatory involvement, an US examination of the affected joint, arthrocentesis of the knee and analysis of synovial fluid. Furthermore, demographic data and clinical history were collected in order to better define the form of CPPD according to the EULAR

task force definitions (1). Particular attention was paid to positive history of acute attacks of arthritis of the knee intended as acute swelling (developed in less than 24 hours) with inflammatory pain. The reference exam for the diagnosis of CPPD and classification of patients was the recognition of CPP crystal by microscopic analysis of the synovial fluid samples. OA was diagnosed at the US examination by detection of characteristic findings as described previously (20, 21). Clinical assessment, US examination and SF analysis were carried out by different operators blind to each other findings.

US examination

The US examination was carried out on both knees in order to evaluate the presence of CPP deposits and only in the symptomatic knee for the assessment of inflammatory features and structural changes. The presence of CPP crystals was evaluated at the level of hyaline cartilage, at the meniscal fibrocartilage, at the quadriceps tendon and at the patellar tendon by a dichotomous score on the presence/absence of CPP crystals following the diagnostic criteria recently established by the OMERACT experts (15).

Regarding the inflammatory changes, the presence of synovitis (*i.e.* synovial hypertrophy and synovial fluid) and power Doppler signal using a semiquantitative score as previously described (20) were assessed.

Structural changes were defined and assessed according to previously published scoring systems regarding femoral-tibial osteophytes and hyaline cartilage thinning (20) by a semiquantitative score ranging from 0 (normal) to 3 (severe damage).

US exams were performed with the patients lying on the examination couch and with high-end US equipment (Esaote MyLab 70XVG, Esaote Italy and Samsung RS80A, Samsung, South Korea) and high frequency (10–18MHz) linear probes. The suprapatellar and parapatellar recesses were evaluated using longitudinal scans with the quadriceps tendon under contraction. The knee menisci were examined with the knee in complete extension and in semi-flexed position (30–45°) sliding with the probe (without lifting it) over the structure under examination from the anterior to the posterior horn. Both transverse and longitudinal scans could be used if considered necessary. Hyaline cartilage was scanned with the knee in maximum flexion for the anterior portion with both transverse and longitudinal scans. The posterior portion of the hyaline cartilage was also scanned with patient lying in prone position.

US examinations were carried out by two different sonographers. Inter-reader agreement for US findings was calculated in previous studies (15, 22) and was found to range from good to excellent depending on the finding to assess.

Arthrocentesis

Knee arthrocentesis was performed with the patient lying on the examination couch and under US guidance. The needle was inserted in the suprapatellar pouch under ultrasound guidance with the in plane technique and the whole amount of liquid was aspirated. If necessary, a second operator could press on the knee in order to push the liquid towards the needle. Double skin disinfection with clorexidine and iodopovidone was performed at the point of injection while the US probe was covered with a sterile membrane and sterile gel was used during the procedure. The whole amount of synovial fluid was registered and a sample was then stored in sterile jar in order to proceed to microscopic analysis.

Arthrocentesis was carried out by the sonographer immediately after the US examination.

Analysis of synovial fluid

SF samples were promptly analysed after arthrocentesis (within 30 minutes) by optical light microscopy according to standard methods (23). In brief, the total and differential white blood cell (WBC) counts were determined using a Bürker counting chamber and pre-stained slides for cell morphology (Testsimplets®), respectively. CPP crystals were identified by morphology and birefringence using compensated polarised light microscopy. A mean of 800 fields were observed before deciding that a sample was negative for CPP crystals.

CPP crystal count

In CPP-positive SFs, a semiquantitative analysis was performed in order to evaluate the total amount of CPP crystals detectable in a single slide. Briefly, a small drop of fresh SF was placed on a glass slide, examined by compensated polarised microscopy (400x), and the number of CPP crystals was recorded for each field. As we considered a total of 800 fields per slide, crystal amount was expressed as number of CPP crystals/800 fields. If the number of CPP crystals exceeded 100 in at least 10 fields, the crystal amount was considered >1000/800 fields as the maximum score.

Determination of inorganic ions in synovial fluid

After examination, SF samples were centrifuged at 3,000 g for 10 min to remove the cells, particulate material, and debris. The supernatants obtained were assessed for the content of the inorganic ions PPi ($P_2O_7^{4-}$), Pi (PO_4^{3-}), Ca^{2+} and Mg^{2+} by fluorometric or colorimetric assays that are commercially available (Sigma-Aldrich, St. Louis, MO, USA). All assays were performed in duplicate.

All SF analyses, inorganic ion determination and ratio between the different ion concentrations in the SF, were carried out by the same operator.

Statistical analysis

Descriptive analyses were performed for all variables and were presented as mean and standard deviation (SD), median and interquartile range (IQR), absolute and relative frequency, according to their type and distribution.

Association between the variables of interest (imaging and laboratory) with OA/CPPD was systematically assessed. Associations of categorical variables or quantitative variables with OA/CPPD were assessed by Chi square or Mann-Whitney test, respectively.

The correlation between imaging and laboratory variables was calculated using Spearman's rank correlation coefficient.

p-values less than 0.05 were regarded as statistically significant. Statistical analysis was performed using STATA v. 11 (STATA Corp., Texas, USA).

Ethics

The study was approved by the local Ethics Committee of the University of Siena and all the patients signed the informed consent before entering the study.

Results

Sixty-seven patients were enrolled in the study, 25 affected by OA (14 males) and 42 affected by CPPD (25 males). There were no significant differences in the two groups of patients regarding age, gender and history of acute attacks of the knee but patients with OA had a significantly higher BMI than patients with CPPD (Table I). Moreover, 7 patients had an acute arthritis at the moment of enrolment and all of them were affected by CPPD.

Regarding the US examination, a significantly higher volume of effusion was identified in patients affected by CPPD as well as a higher degree of synovitis. The difference was significant also after exclusion of the patients with acute arthritis at the time of enrolment. On the other hand, there were no significant differences between the groups regarding structural changes of the knee as assessed by US and the PD signal (Table II).

At arthrocentesis, a significantly higher amount of SF was aspirated from patients affected by CPPD, confirming the US data. Also, in this case, a statistical significance was preserved when patients with acute arthritis were excluded from the analysis. The correspondence between US grade of effusion and the volume of SF aspirated at arthrocentesis was calculated and is presented in Figure 1.

At the SF analysis, the WBC count was significantly higher in patients with CPPD that presented also a higher percentage of PMN and a lower percentage of monocytes than patients with OA. This statistically significant result was preserved also after removing from the analysis the 7 patients with acute flare that present very high numbers of WBC countv (24). In Table I the SF analysis data does not include patients with acute attack. Regarding the inorganic ion concentration assessment, patients with CPPD presented a higher concentration of PPi and a lower concentration of Mg in the SF than patients with OA alone, however, statistical analysis did not reveal any statistically significant difference between the two groups for any of the elements examined (Table III). The total number of crystals in the SF, correlated with larger effusion, a higher grade of synovitis in US and a higher total WBC count and PMN percentage in the SF (Table IV).

Table I. clinical characteristics and SF analysis results in the two groups.

	OA	CPPD	*p*-value
n.	25	42	
Male, n (%)	14 (56%)	25 (59%)	0.77
Age (years), mean (SD)	71.8 (±9.5)	69 (±12.5)	0.67
BMI, mean (SD)	28.43 (±2.9)	26.06 (±3.6)	<0.01
History of acute arthritis, n (%)	11 (44%)	31 (74%)	0.13
Volume of synovial liquid (ml), mean (SD)	7 (±5.9)	14 (±13)	<0.01
WBC count (n/mcl), mean (SD)	448 (±849)	1395 (±2275)	<0.001
PMN (%), mean (SD)	1 (±3)	8 (±14)	<0.001
Monocytes (%), mean (SD)	97 (±3)	89 (±14)	<0.001
Linfocytes (%), mean (SD)	2 (±2)	3 (±4)	0.62

Patients with acute flare of CPPD (7) were considered outliers and were not included in the analysis presented in this table regarding the WBC count and percentages of cells.
BMI: body mass index; WBC: white blood cells; PMN: polymorphonucleates.

Table II. Ultrasound findings in the two groups of patients.

US Finding	Score	OA n=25)	CPPD (n=42)	*p*-value
Joint effusion, n (%)	0	1 (4)	2 (5)	
	1	16 (64)	10 (24)	0.01§
	2	6 (24)	24 (57)	0.007*
	3	2 (8)	6 (14)	
Synovitis, n (%)	0	11 (44)	8 (19)	
	1	9 (36)	20 (48)	0.08§
	2	5 (20)	14 (33)	0.04*
	3	0 (0)	0 (0)	
PD, n (%)	0	18 (72)	24 (57)	
	1	6 (24)	13 (31)	0.38§
	2	1 (4)	5 (12)	0.18*
	3	0 (0)	0 (0)	
Femural-tibial OA, n (%)	0	0 (0)	2 (5)	
	1	5 (20)	8 (19)	0.44§
	2	14 (56)	17 (40)	0.68*
	3	6 (24)	15 (36)	
Femural-patellar OA, n (%)	0	1 (4)	4 (9)	
	1	5 (20)	12 (29)	0.66§
	2	12 (48)	16 (38)	0.31*
	3	7 (28)	10 (24)	

PD: Power Doppler US. §Pearson Chi square test (used to test overall differences across all groups treating variable as categorical); *Two-sample Wilcoxon rank-sum (Mann-Whitney) test (used to test overall differences across all groups using ranks).

Discussion

Even if CPPD is a very common arthropathy in the elderly (17, 25, 26), there is no specific treatment aiming to crystal dissolution or at least to reduce inflammation and joint damage in the affected patients. Pathogenetic pathways that lead to crystal deposition and disease progression are not clear and the lack of animal or *in vitro* models able to reproduce the joint conditions in CPPD, make it an hostile disease to study. Furthermore, the multitude of clinical patterns of the disease (27) can lead to misdiagnosis and inappropriate treatment of these patients. The diagnosis of CPPD is basically based on the SF analysis that may reveal typical crystals at polarised

Table III. Concentrations of inorganic ions (mean values and SD) in the synovial fluid expressed in micromols.

	Ca	Mg	Pi	Ppi	Ca/Ppi	Mg/Ppi	Pi/Ppi	Ca/Pi	Ma/Pi	Ca2/Mg2
OA	2153.18±442.66	709.17±132.41	4406.65±920.92	6861.62±19194.31	1.191±0.506	0.403±0.205	2.378±1.009	0.497±0.095	0.166±0.038	3.111±0.788
CPPD	2228.60±640.29	686.26±140.83	4714.54±1502.35	8498.716±18367.37	1.30±1.038	0.407±0.313	2.531±2.112	0.501±0.148	0.157±0.50	3.308±0.831
p	0.99	0.39	0.66	0.71	0.81	0.57	0.87	0.77	0.34	0.35

There were no statistically significant differences between the two groups.

light microscopy (1) but in many cases SF is not available for analysis. US has demonstrated to be a reliable and accurate exam for identifying CPPD (12, 15) and for assessing inflammation and joint damage (20).

In a recent paper, Abhishek *et al*. aimed to identify the unmet needs in CPPD involving the members of the G-can, experts in microcrystalline arthropathies. According to their research agenda, exploring the association between OA and CPPD is a priority and "understanding unique characteristics of patients with knee OA with and without CPPD should lead to improved insight into OA pathogenesis, and how CPPD contributes to OA, as well as identification of potential therapeutic targets" (19).

In our study, US revealed a higher amount of effusion and a higher grade of synovitis in patients affected by CPPD, indicating a higher degree of inflammation. The higher amount of SF was also confirmed at joint aspiration. On the other hand, no statistical difference was found regarding osteophytes and cartilage damage in the knees of the two groups. However, considering that patients with OA had a higher mean age and a significantly higher BMI it could be reasonable to expect a higher degree of joint changes in the OA group. An explanation could be that even if US may capture many aspects of joint damage, it misses some very important changes such as joint space narrowing, subchondral bone sclerosis and bone attrition that are also important in defining joint damage both in OA and CPPD (2, 4, 28). Longitudinal studies, with implementation of other imaging techniques that provide a more accurate evaluation of bone damage, could be useful in order to assess if patients with CPPD have a different progression of joint changes compared to OA.

Table IV. Correlation between number of crystals in the synovial fluid and polymorphonucleates count, volume of effusion and ultrasound synovitis.

	Number crystals *vs*. PMN count	Number crystals *vs*. effusion	Number crystals *vs*. synovitis
Spearman r	0.64	0.36	0.32
p (two-tailed)	<0.0001	0.002	0.008

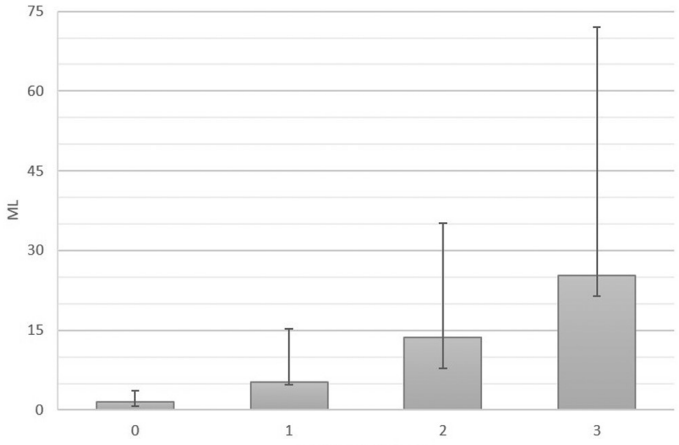

Fig. 1. Correspondence between grade of US effusion and synovial fluid aspirated at arthrocentesis. On the x-axis is represented the US grade of effusion in a semiquantitative score (0: absent, 1: mild effusion, 2: moderate effusione, 3: severe effusion) while on the y-axis is represented the volume (mean and SD) of effusion aspirated during ultrasound guided arthrocentesis.

At SF analysis, patients with CPPD had a higher WBC count and a higher percentage of PMN than patients with OA alone. This could be due to the presence of a higher number of proinflammatory cytokines in the SF of patients with CPPD as demonstrated by previous studies (6, 7). In detail, Il-8 a direct neutrophil chemotaxin but also inducer of production of other neutrophil chemotaxins, has been found in SF of patients with CPPD and can be produced by cells stimulated with CPP crystals *in vitro* (29, 30). Thus, it is not surprising that patients with CPPD have a higher SF WBC count. Furthermore, Il-8 could be a target for future treatments in CPPD.

A very interesting result of this study regards the inorganic ion concentration in the SF of the two groups. In our cohort, there were no significant differences between the groups regarding the concentrations of Ca^{2+}, Mg^{2+}, Pi and PPi even if a trend indicating higher concentrations of PPi and Ca^{2+} in patients with CPPD was observed. Calculating the ratios between Ca^{2+} and Mg^{2+} and Ca^{2+} and PPi also did not provide statistically significant differences. This finding is in accordance with previous studies that demonstrated that higher PPi levels are also present in patients with OA if compared to normal subjects (9, 31) but partially in contrast with others that found a statistically significant difference between patients with OA and CPPD (32). However, in the study by Pattrick *et al*. (32), patients with CPPD were older than patients with OA alone and probably with a higher grade of OA. Furthermore, it is not clear how did the authors get the synovial fluid of normal subjects and if patients affected

by RA had also OA (x-rays were not performed in these patients in order to rule out the coexistence of OA).

Finally, it seems that the number of CPP crystals in the SF influences directly the amount of joint effusion and synovitis. In a previous study it was suggested that patients with a lower amount of CPP deposits in cartilage and fibrocartilage of the knee had a higher amount of acute attacks and inflammation in the joint (16). Probably, the passage of crystals from the cartilage/fibrocartilage in the synovial space could explain both the increased amount of liquid and inflammation and the reduced quantity of deposits in US imaging. It is plausible indeed that deposition of crystals could be asymptomatic as long as CPP do not pass in the synovial space. Once the shedding occurs, inflammatory pathways are triggered, and clinical symptoms of inflammation appear.

A limitation of our study is that we only included patients with a painful knee and joint effusion, missing all patients with or without pain but without joint effusion. However, this was the only way to address our research question regarding the differences in terms of inflammation between the two diseases. Including in the cohort patients with OA and CPPD but without symptoms or with symptoms but without signs of inflammation probably would not allow the identification of any differences between the two groups in a transverse study as the one we performed. A long-term longitudinal study including patients with both pathologies and at any phase of the diseases would allow probably to address better the differences in terms of joint damage characteristics/progression and inflammatory involvement of joints. This first exploratory study constitutes a solid base to proceed to this next step.

In conclusion, in our cohort, patients with CPPD have a greater degree of inflammation compared to patients with OA alone as testified by both US examination and SF analysis, highlighting the need for specific and probably long term anti-inflammatory treatment in these patients. Furthermore, inflammatory manifestations seem to be directly linked to the amount of crystals in the SF. On the other hand, structural damage as assessed by US and with the limitations of the technique, does not seem to be different than the one of patients with OA and knee pain. Inorganic ion concentration in the SF does not appear to be significantly different between the two groups of patients. According to our data, it is plausible that different pathogenetic pathways are involved in CPPD and OA leading to a greater amount of inflammation in the first group. The similar concentration of inorganic ions in the SF indicates that the trigger for CPP formation is not in the SF but in other tissues such as the extracellular matrix of hyaline cartilage and fibrocartilage and that SF concentrations reflect only part of the changes that occur within the different joint structures, accordingly with previous studies (31). According to our data, it is reasonable to assume that OA and CPPD may not be two "associated" diseases as generally believed but could be two distinct diseases with a common risk factor, that is aging, and thus involving the same group of patients. US can be useful in identifying crystals and assessing inflammation in patients with suspected CPPD and/or OA and could be a feasible and reliable tool to use in longitudinal studies in order to better understand CPPD disease evolution over time and its relationship with OA.

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