Diagnostic accuracy of ultrasound, conventional radiography and synovial fluid analysis in the diagnosis of calcium pyrophosphate dihydrate crystal deposition disease

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Abstract

Objective

To assess the diagnostic performance of ultrasound (US), x-rays, and microscopic analysis of synovial fluid (SF) for calcium pyrophosphate dihydrate crystal deposition disease (CPPD) using histology as a reference standard

Methods

We enrolled consecutive patients with osteoarthritis waiting to undergo knee replacement surgery. Each patient underwent US of the knee, focusing on menisci and the hyaline cartilage, the day before surgery. During surgery, SF, menisci and condyles were retrieved and examined microscopically. For the meniscus and cartilage microscopic analysis, 8 samples were collected from each specimen and knee radiographs, performed up to 3 months before surgery, were also assessed. A dichotomous score was given for the presence/absence of CPP for each method. Microscopic findings of the specimens were considered the reference standard. All the procedures followed were in accordance with the ethical standards of the local responsible committee.

Results

42 patients (14 males) were enrolled. All patients underwent US, 34 had eligible radiographs and 32 had SF analysis. 25 patients (59.5%) were positive for CPP at US, 15 (44.1%) at X-ray and 14 (43.7%) at SF. Sensitivity and specificity values were 96% and 87% for US, 75% and 93% for radiography and 77% and 100% for SF respectively. There were no statistically significant differences between the diagnostic performance across single tests.

Conclusion

US proved to be at least as accurate as SF analysis for the diagnosis of CPPD. US, which is feasible and harmless, could be considered the first exam of choice for CPPD diagnosis.

Key words

ultrasound, x-rays, synovial fluid, sensitivity and specificity, chondrocalcinosis, calcium pyrophosphate dihydrate deposition disease

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Introduction

Calcium pyrophosphate dihydrate crystal (CPP) deposition disease (CPPD) is one of the most frequent arthropathies among the elderly. In previously published papers, the prevalence of CPPD varied largely depending on the patients' age and diagnostic method used. By using x-rays, the prevalence ranged between 3.7% in younger patients (55-59 years old) and 21% in the elderly (over 85 years old) (1-4). When using synovial fluid (SF) microscopic examination as a diagnostic tool, the prevalence of CPPD rose to 25–43% (5-7). There is no available data on the incidence and on the socio-economic impact of this disease. Further, other gaps of knowledge exist from diagnosis, to prognosis and treatment.

The first step to try to fill these gaps is to have an accurate and feasible diagnostic tool to reliably identify patients with CPPD. In the past, the diagnosis of CPPD has been mainly based on x-rays and SF microscopic analysis as requested by the McCarty's diagnostic criteria (8). Recently, the EULAR task force published new recommendations on the terminology and diagnosis of CPPD.

Regarding the terminology, the experts defined CPPD as the umbrella term for all instances of CPP crystal occurrence, ranging from the completely asymptomatic form (with or without osteoarthritis) to chronic arthritis. Further, in the asymptomatic form of CPPD, the experts also included the term chondrocalcinosis (CC), the identification of CPP crystals upon imaging.

Regarding the diagnostic guidelines for CPPD, the EULAR experts indicated that SF analysis could be sufficient to establish a diagnosis and that radiographs alone are not sensitive enough to confirm a diagnosis. Furthermore, they acknowledge the utility of ultrasound (US) in the diagnostic process (9). Despite the important introduction of US in the guidelines, there is still great uncertainty with regard to CPPD diagnosis, as no data is available to determine the accuracy of the tests that are currently used for this purpose (10). Thus, the aim of this study was to evaluate the diagnostic accuracy of US, microscopic analysis of SF and x-rays in the diagnosis of CPPD of the knee, using the presence of CPP in its' tissues as revealed by microscopy as reference standard. On this basis, the objective of this study is not to classify patients according to the form of clinical presentation (asymptomatic, acute or chronic arthritis) but only according to the presence or absence of CPP crystals in the joints. In order to avoid terminology confusion, in this paper we will use the term of CPPD also for the imaging tests, assuming that calcifications seen on x-rays are always due to CPP crystals.

Patients and methods

All patients signed an informed consent for the participation in the study. All the procedures followed were in accordance with the ethical standards of the local responsible committee for human experimentation.

We enrolled the first 42 consecutive patients with osteoarthritis (OA) who were waiting for total knee replacement (TKR) surgery at the University Hospital of Siena, from June 2013. The sample size was calculated for US (11-13), considering a prevalence of the disease of 40% (5), a precision of 0.15 and a confidence level of 95%. The orthopaedic surgeon that performed the TKR has 20 years experience.

All patients had an US examination of the knee being subjected to surgery, by a rheumatologist with 15 years experience in musculoskeletal US, the day before surgery. US scans were performed at the level of the medial and lateral meniscus, with the knee completely extended, semi-flexed and completely flexed, without raising the probe all the way along the medial and lateral rim and at the level of the hyaline cartilage of the femoral trochlea with the knee completely flexed and with longitudinal and transverse scans. An increase of dynamic compression was used in some cases in order to enhance the contrast of the image and better isolate CPP deposits from the surrounding structures. No other joint structures were examined and the sonographer did not ask the patients any questions. The sonographer gave a dichotomous score based on the absence/presence of CPP deposits in

the meniscus and cartilage, according to previously published US diagnostic criteria (11-13) and personal experience (14, 15). An Esaote Mylab 70XVG (Esaote, Genoa - Italy) scanner equipped with a 7-13MHZ linear probe was used for this study.

X-ray files were saved by the surgeon in a digital file format (DICOM format where possible, otherwise a high quality .jpeg format was used). All x-rays were performed in conventional anterior-posterior, lateral projections and in the standing position, as requested in routine clinical practice for standard assessments of knee OA. These files were then evaluated by an independent radiologist with 10 years experience in musculoskeletal radiology, in random order and blinded to other findings and clinical data. The radiologist scored x-ray images for the absence/presence of calcifications at the levels of the medial and lateral meniscus and the cartilage. Only x-rays performed up to 3 months before surgery have been considered in this study.

Synovial fluid was collected with a syringe by the surgeon during arthrotomy, put in a sterile container and kept in a refrigerator at 4°C until it was examined, within 36–48 hours from surgery. This is considered an acceptable time for a reliable CPP crystals identification (16). All synovial fluids were examined by the same examiner (20-years experience in SF analysis), blind to other findings, on wet preparations on slides obtained by placing a tiny drop of carefully shaken fluid onto a degreased slide that was then covered with a coverslip. Each slide was observed under transmitted light microscopy and compensated polarised microscopy. At least 30 adjacent microscopic fields of each slide were scanned. Crystals with a parallelepiped or rhomboid shape and weak birefringence with positive elongation were considered to be CPP crystals.

During surgery, anatomical specimens of the knee were collected and we chose to retrieve both menisci and the hyaline cartilage of the trochlea, the main sites of CPP deposition, as previously described (14). The specimens were then delivered to a rheumatologist expert in the microscopic diagnosis of microcrystalline arthritides. During this stage, slides of the knee tissues were prepared and observed under microscopy (direct and compensated polarised light) for the research of CPP in the tissues. Eight random samples were taken from the meniscus, both medial and lateral. In the case of the cartilage, four random samples of the cartilage were taken and analysed. The microscopic criteria for the identification of CPP crystals were the same as those used for SF analysis.

The reference standard for the final diagnosis of CPPD was the presence of CPP crystals of the knee tissues at microscopic examination. Considering the fact that CPP crystals are primarily formed within the cartilage and fibrocartilage structures of the joint (8), microscopic analysis of these tissues should be the best way to identify crystals before they are liberated into the SF (as with SF analysis) or before they reach dimensions that can be detectable with imaging (*i.e.* US or x-ray).

For analysis purposes, US was considered positive for CPPD even when only one of the structures examined was positive. X-rays were considered positive if the radiologist gave a positive scoring at one of the joint spaces examined. When test combinations were analysed, the positivity to at least one test was considered as positive, while the absence of crystals at all the tests was required for a negative score. A flow-chart of the study is reported in Figure 1.

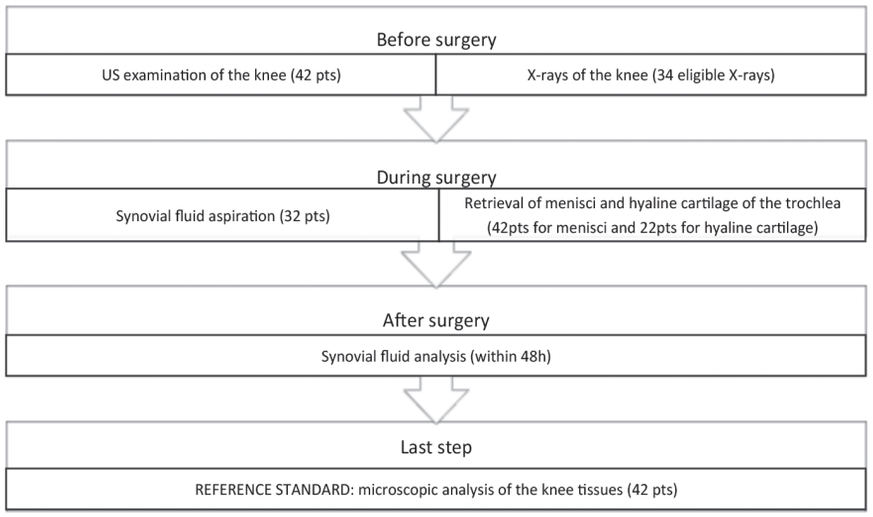

Fig. 1. Study work-flow. For more details on the various steps please see the patients and methods section.

Prevalence, sensitivities, specificities, positive and negative predictive values, area under the curve (AUC) for US, X-rays and SF aspiration were calculated with exact 95% confidence intervals using the Stata 11.0 statistical software package (Stata Corp., College Station, Texas). Comparisons of sensitivity and specificity between tests or test combinations were evaluated by McNemar test, and differences between AUC using the roccomp command in Stata.

Results

Of the 42 patients enrolled in the study, 14 were men and 28 women. The mean age was 74 years (SD ±8.4) and the mean BMI was 27.8 (SD ±2.9). All patients underwent an US examination of the knee before surgery, 32 of them had joint effusion that permitted microscopic analysis of the synovial fluid and 34 had knee radiographs performed up to 3 months before surgery.

Upon microscopic analysis of the specimens, 26 patients (62%) had at least 1 positive slide for CPP crystals. Hyaline cartilage of the knee was examined in only 22 patients as the remaining had large areas of bone exposure due to severe osteoarthritis and cartilage samples could not be collected. All menisci, medial and lateral, were retrieved except 1 medial meniscus due to a previous medial meniscectomy. 21 patients were positive at the medial meniscus, 25 at the lateral meniscus and 10 (out of 22 examined) at the hyaline cartilage

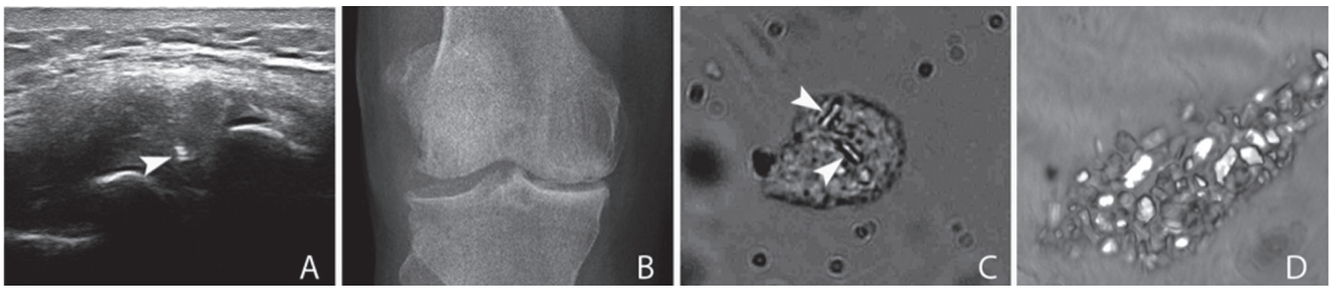

Fig. 2. A case of a patient with positive US, SF and microscopic examination and negative x-rays. **A**: US examination of the medial meniscus; **B**: x-rays of the knee acquired before surgery; **C**: direct light microscopic examination of the synovial fluid; **D**: aggregate of CPP crystals at the microscopic examination of the medial meniscus in polarised light microscopy. Arrowheads: CPP crystals.

level. 21 patients showed CPP crystals at both menisci.

Upon US examination, 20 patients presented CPP crystals at the medial meniscus and 23 at the lateral. 16 patients were positive at both menisci, 6 only at the medial, and 4 only at the lateral. Only 39 patients were given a score for the hyaline cartilage because it was very thin in 3 patients due to OA, hampering a correct assessment. Five of them had CPP deposits upon US. Compared to microscopic examination, US had a sensitivity and specificity of 90% and 100% at the medial meniscus, 80% and 87% at the lateral meniscus and 70% and 100% at the cartilage level.

Upon x-ray, 10 patients presented calcifications at the medial meniscus level, 13 at the lateral meniscus and 2 at the hyaline cartilage as detected at the lateral projections. Sensitivity and specificity values for each site were 53% and 94% for the medial meniscus, 60% and 93% for the lateral meniscus and 12.5% and 100% for the hyaline cartilage.

At SF analysis 14 out of 32 patients were positive for CPPD.

Considering the whole knee joint, US correctly classified 25 out of 26 patients as affected by CPPD and 14 out of 16 patients as unaffected. X-rays correctly classified 15 out of 20 patients affected by CPPD and 13 out of 14 unaffected patients, while SF analysis correctly classified 14 out of 18 CPPD patients and all of the unaffected patients. In Figure 2 is represented by way of example, a patient affected by CPPD, with positive US and SF analysis and negative x-rays.

Summarised in Table I are the overall values of sensitivity, specificity (with CI 95% in parenthesis), positive predictive value (PPV), negative predictive value (NPV) and accuracy, considering as positive a patient that had at least one positive finding at the sites examined with each method. The statistical analysis did not find any significant difference between the performance of the single tests for either sensitivity or specificity. However, comparing the tests performance with McCarty's criteria (definite diagnosis of CPPD when a patient is positive in both SF and x-rays), US was more sensitive in the identification of CPPD (96.2 (80.4–99.9) *vs*. 47.1 (23.0–72.2); p=0.025), while there were no statistically significant differences regarding specificity (96.2 (80.4-99.9) *vs*. 100 (79.4–100); p=0.157) (Table II). Further, by examining the area under the curve for the three techniques, US was the only exam that demonstrated a significantly higher performance compared to the McCarty criteria (AUC 0.92 (0.83–1.00) *vs*. 0.74 (0.61–0.86). (Tables III).

Discussion

As defined by the EULAR task force of experts (9), CPP deposition disease can present a variety of clinical manifestations ranging from the asymptomatic form to the well-known acute attack generally defined as "pseudogout". Consequently, CPPD is not only acute arthritis but it may have various clinical presentations, both with and without association to OA, which could complicate the diagnosis. Further, periarticular manifestations seem to be also more frequent in patients affected by CPPD compared to other crystal related arthritides such as gout (17). For these reasons, it is important for the clinician to establish if CPP deposition is present in the patient's joints, independent of the phase of the disease or the clinical picture of the patient at that given moment. CPPD is probably one of the most common diseases among the elderly, reaching a prevalence peak of over 40% in selected patients with knee pain (5). In our study, the prevalence of CPPD in patients undergoing total knee arthroplasty was 62% and this can be explained by the highly sensitive method used to determine the presence of the disease. Both our and the previous studies (5) have dealt with elderly patients in the final stage of osteoarthritis thus not necessarily reflecting the general popula-

Table I. sensitivity and specificity with 95% confidence intervals (CI), positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of the various methods *versus* the reference standard. SF: synovial fluid, in parentheses: numerators and denominators for all percentages provided.

	Sensitivity [CI 95%]	Specificity [CI 95%]	PPV	NPV	Accuracy
US	96% [±0.07] (24/25)	87% [±0.16] (14/16)	92% (24/26)	93% (14/15)	93% (38/41)
X-rays	75% [±0.18] (15/20)	93% [±0.13] (13/14)	94% (15/16)	72% (13/18)	82% (28/34)
SF analysis	77% [0.19] (14/18)	100% [0] (14/14)	100% (14/14)	78% (14/18)	88% (28/32)

Table II. P-values of the comparisons (McNemar test) of the sensitivity and specificity of the exams and combinations. In grey the statistically significant values. In this table, when considering a pair of exams, a patient is considered positive if he is positive in both exams and negative if at least 1 exam is negative.

	US	X-rays	SF	US+X-rays	US+SF	X-rays+SF
Sensitivity						
US	-					
X-ray	0.102	-				
SF	0.083	0.414	-			
US+X-ray	**0.025**	0.317	0.654	-		
US-SF	0.083	0.414	N/A	0.654	-	
X-ray+SF	**0.004**	**0.045**	0.157	0.083	0.157	-
US+X-ray+SF	**0.004**	**0.045**	0.157	0.083	0.157	N/A
Specificity						
US	-					
X-ray	0.563	-				
SF	0.317	0.317	-			
US+X-ray	0.157	0.317	N/A	-		
US-SF	0.317	0.317	N/A	N/A	-	
X-ray+SF	0.157	0.317	N/A	N/A	N/A	-
US+X-ray+SF	0.157	0.317	N/A	N/A	N/A	N/A

US: ultrasound; SF: synovial fluid analysis; N/A: not applicable.

Table III. Comparison of the three techniques and their combination with the McCarty criteria for definitive diagnosis. According to these criteria a patient is considered definitely affected by CPPD when he is positive both in SF and x-rays. In this table, when considering a pair of exams, a patient is considered positive if he is positive in both exams and negative if at least 1 exam is negative.

	n	Sensitivity (95%CI)	Specificity (95%CI)	AUC (95%CI)	*p*-value
US	42	96.2 (80.4-99.9)	87.5 (61.7-98.4)	0.92 (0.83-1.00)	0.022
X-ray	34	75.0 (50.9-91.3)	92.9 (66.1-99.8)	0.84 (0.72-0.96)	0.200
SF	32	77.8 (52.4-93.6)	100 (76.8-100)	0.89 (0.79-0.99)	0.141
US-X-ray	36	70.0 (45.7-88.1)	100 (79.4-100)	0.85 (0.75-0.95)	0.064
US-SF	33	77.8 (52.4-93.6)	100 (78.2-100)	0.89 (0.79-0.99)	0.141
X-ray+SF	33	47.1 (23.0-72.2)	100 (79.4-100)	0.74 (0.61-0.86)	Reference

p-value of the comparison of the AUC of single tests or combinations using McCarty criteria as comparator.

tion. Unfortunately, there are no studies in the general (unselected) population aimed at assessing the prevalence and incidence of this disease, which can be ascribed in part, to the lack of validated and non-invasive diagnostic methods. The objective of this study was to assess the diagnostic accuracy of three different methods within our population and a theoretically expected higher than usual prevalence of CPPD was optimal for this purpose.

US demonstrated high values of sensitivity and acceptable values of specificity, in accordance with previous studies (11, 12, 13, 18). A similar study has recently been published by Gutierrez *et al*. (19). In that study the authors showed sensitivity and specificity values of 90.5% and 100% respectively for the US detections of CPPD at the meniscus and 59.4% and 100% at the hyaline cartilage. The main difference with our study is that they used the SF analysis as the reference standard for diagnosis whereas we used a more strict reference test. Furthermore, in our study we have presented data on a patient basis and not according to the structure under examination (meniscus/cartilage) in order to make them readily understandable and more compliant with real life.

Radiography demonstrated low sensitivity and high specificity. The sensitivity of x-rays is low but unexpectedly, it is very similar to that of synovial fluid analysis. This is the first time that the two techniques have been directly compared, as the synovial fluid analysis is usually considered as the gold standard (23, 24) and this could influence the x-ray results. The lower resolution of traditional x-rays compared to US, the flattening of the image and the body composition could be among the factors that influence the diagnostic accuracy of x-rays.

Synovial fluid analysis demonstrated an absolute specificity but a low sensitivity. In this cohort, none of the patients had an acute pseudo-gout attack at the time of enrolment and more specifically the main finding was of few extracellular crystals in the majority of patients. Previously, Martìnez Sanchiz and Pascual demonstrated that CPP crystals are present in the synovial fluid of patients affected by CPPD also in non-inflammatory phases and independent from a previous pseudogout crisis (22). However, in that study, all patients had a "longstanding" disease and x-rays were negative in only 4 out of 72 patients. The main differences with this study, where we observed the absence of CPP crystals in the synovial fluid of affected joints in some cases, is that their study was not blinded and that our patients could be in an "early" phase of CPPD even if in a late phase of osteoarthritis. Therefore, according to our results, CPP crystals are not always present in the SF of affected joints and this could be explained by the shedding theory and probably by the stage of the disease. It could be of interest, to further investigate the role of crystals in the SF of joints that do not present as pseudogout in order to assess if they play a role in subclinical inflammation and joint damage progression.

The statistical analysis demonstrated that, in spite of the apparently evident difference in the sensitivity values, there was no statistical difference between the three examinations. The same result was also observed for specificity values in spite of the 100% specificity of SF analysis. This could probably be due to the relatively small number of patients that performed all of the exams (only US was carried out for all patients) and secondly because of the high diagnostic accuracy of all

the tests. According to these results, the three techniques could be considered equivalent for CPPD with only one being possibly sufficient to reach a diagnosis. Considering the global performance of the tests (AUC) and in comparison to McCarty's criteria for diagnosis, US was the only exam that achieved a statistically better performance. This is firstly due to its higher sensitivity values and secondly to the fact that McCarty's criteria requires both SF and x-rays to be positive in order to confirm a diagnosis reducing sensitivity. Further, the single tests did not demonstrate any statistical difference in their global performance (AUC) when compared to McCarty's criteria. In addition, single tests seem to obtain better sensitivity values than paired tests (in case of paired tests a patient was considered affected by CPPD when both exams were positive) and similar specificity values. This data suggests that there is no need to perform a second examination in cases of positivity of the first, in clinical practice.

This study presents some limitations. Regarding x-rays, we chose to enrol only the patients that had performed an examination within 3 months before surgery. We preferred not to excessively penalise the sensitivity of this test as the other tests were performed simultaneously. Further, radiological assessment of CPPD was sometimes based on exported jpeg files and not DICOM files, a fact that could create some false results. However, the radiologist involved in the evaluation of the radiographs only encountered some difficulty in two of the cases. The excellent specificity results support the hypothesis that jpeg files are of sufficient quality for assessing CPPD. Previous studies on SF analysis, raised some concerns about its reliability (10, 20). On the other hand, inter-reader agreement values for the presence of CPPD deposits in the knee as identified by US between expert sonographers were satisfactory, as demonstrated in a previous study (19). In the study by Gutierrez *et al*. (19) kappa values between two observers, for hyaline cartilage and menisci were also good (0.72 and 0.68 respectively). In this study, unfortunately, it was not possible to assess inter-reader agreement neither for US nor for the other techniques without disrupting the blind process of the operators. Intra-reader reliability was not assessed for US because of the study design (patients underwent knee surgery some hours after US). Finally, the three tests considered in this study, currently test different aspects of the same disease. X-rays and US are exams that evaluate the presence of macroscopic aggregates of CPP in cartilage and fibrocartilage while SF analysis tests the presence of microscopic crystals in a biologic fluid. Considering that the pathogenesis and the natural history of the disease are not yet clarified, it is difficult to compare these three exams. However, at present, they represent our diagnostic tools in daily clinical practice, and our study can help to increase understanding of their role in this setting.

In summary, we demonstrated that US is at least as accurate as synovial fluid microscopic analysis for the diagnosis of CPPD. Considering the three techniques under examination, US is the most feasible as it can be carried out directly by the clinician, it can be diagnostic even when SF is not present and is probably the most patient-friendly. On the other hand, SF analysis can be performed only when effusion is present and can be demanding when deep or small joints are affected but thanks to its absolute specificity, it can confirm a diagnosis in difficult cases. The role of x-rays, in consideration of their intrinsic characteristics and in the light of our findings has to be defined. Further studies are needed in order to confirm this data in other joints. In conclusion, if you have any doubts regarding the presence or absence of CPPD … you could let US help you!

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