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Diagnostic value of ultrasound in calcium pyrophosphate deposition disease of the knee joint

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SUMMARY

Objective: To assess the diagnostic performance of ultrasound (US) for calcium pyrophosphate deposition (CPPD) at the level of menisci, hyaline cartilage (HC), tendons, and synovial fluid (SF) of the knee, and to examine inter- and intra-observer reliability.

Design: We consecutively included patients with knee effusion over a 2-year period (43 patients with CPPD and 131 controls). All patients underwent SF analysis, conventional radiography (CR), and US examination using the Outcome Measures in Rheumatology (OMERACT) definition of the US characteristics of CPPD. Two independent operators performed the US, and inter-observer agreement was calculated. Intra-observer agreement was examined with static images obtained for all enrolled patients.

Results: US revealed calcium pyrophosphate (CPP) deposits in menisci, HC, and tendon more frequently in patients with CPPD than in control patients. The presence of US CPP deposits in SF was not significantly different between the two groups. Combined US evaluation of the three components (menisci, HC, and tendon) showed the best diagnostic performance. The sensitivity and specificity for US evaluation of the three components were 74.4% and 77.1%, respectively, while for CR evaluation, the sensitivity and specificity were 44.2% and 96.9%, respectively. Inter- and intra-observer agreement were excellent for medial ($\kappa = 0.930$, 0.972) and lateral menisci ($\kappa = 0.905$, 0.942), HC ($\kappa = 0.844$, 0.957), and SF ($\kappa = 0.817$, 0.925). Tendon showed fair inter-observer ($\kappa = 0.532$) and good intra-observer reliability ($\kappa = 0.788$).

Conclusions: Based on the OMERACT definition, US demonstrated better diagnostic capacity than CR to diagnose CPPD, with excellent reliability. Combined evaluation of menisci, HC, and tendon showed the best diagnostic accuracy.

Introduction

Calcium pyrophosphate deposition (CPPD) disease is the third most common inflammatory arthritis caused by calcium pyrophosphate (CPP) crystals[1]. CPPD occurs primarily in the articular tissues, most commonly in the fibrocartilage and hyaline cartilage (HC). The gold standard for diagnosis of CPPD is the identification of CPP crystals that appear as positively birefringent, rhomboid-shaped crystals in the synovial fluid (SF) of an affected joint[2]. Despite its low sensitivity, conventional radiography (CR) is the most widely used imaging method. It is a fast, simple, applicable and inexpensive imaging modality of joints evaluation and provides support for the diagnosis of CPPD by showing chondrocalcinosis (CC). CR has clear advantages over ultrasound (US) in the visualization of deeper structures. Although US could not be a replacement for CR, a complementary to CR is US, a promising imaging technique for CPPD. There are several studies showing its higher sensitivity than CR[3]. And the feasibility of US in CPPD is emphasized in the European League Against Rheumatism (EULAR) recommendations for CPPD[4].

US is a non-invasive and convenient diagnostic method, but some limitations remain for its clinical application in CPPD. A recent systematic review has underlined the need for a universally accepted and reliable definition for US features of CPPD[5]. Different descriptions of CPP deposits in various studies make it difficult to compare the US results and perform US in clinical practice. To solve

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this issue, the Outcome Measures in Rheumatology (OMERACT) US subtask force published a new definition for CPPD at the knee level[6].

The advantage of using US is that a wide spectrum of abnormalities in various structures, including articular cartilage, synovial tissue, joint effusion, and tendon, can be evaluated[7]. However, the assessed structures in most US studies were limited to fibrocartilage and HC[8–12]. Therefore, the question remains whether the US features of SF and tendon contribute to the differential diagnosis of CPPD. Another issue is the reliability of US in CPPD especially in the level of SF and tendon. Five studies have evaluated the inter-observer agreement only at the HC and of the fibrocartilage of the knee[11–15]. Although one study has assessed the inter- and intra-observer agreement at the level of HC, fibrocartilage, SF and tendon of the knee, only four patients with CPPD were included in the patent based exercise[6].

In this study, we aimed to assess the diagnostic performance of US for CPPD in menisci, HC, tendon, and SF of the knee using the OMERACT definition. We also examined the intra- and inter-observer reliability in the US assessment of CPPD.

Methods

Patients and study design

We consecutively included patients with knee effusion on clinical examination who underwent SF analysis in a prospective single-centre study. SF aspiration was performed in case of monoarthritis (chronic or acute), suspicion of joint infection or crystal-induced arthritis, or monoarthritis in patients with chronic polyarthritis. All patients were recruited during a 2-year period in the Rheumatology Department of Konkuk University Medical Center. Exclusion criteria were age <18 years, previous knee surgery or trauma, corticosteroid injection within the previous 3 months, and refused or failed arthrocentesis[11].

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board for Human Research, Konkuk University Medical Center (KUH1010879). Written informed consent was obtained from all participants.

SF analysis

SF was analysed using a compensated polarizing microscope to validate the presence of CPP crystals within 4 h of aspiration. Polarized light microscopy was performed by a 13 year specialist of laboratory medicine who was blinded to all imaging and clinical data. Crystals with a parallel epipedic or rhomboid shape and weak birefringence with positive elongation were considered to be CPP crystals. Alizarin red S staining was also performed to detect basic calcium phosphate (BCP) crystals. CPPD was diagnosed by identifying CPP crystals in SF in accordance with the EULAR recommendations for CPPD[4]. Patients who did not have CPP crystals in SF were considered as controls.

Conventional radiographic assessment

All patients underwent radiography of the knee in both standing anteroposterior and lateral views. The radiographic images were analysed by an experienced radiologist with 10 years of experience in musculoskeletal radiology. The radiologist was blinded to both clinical and US images, and assessed the presence of cartilage and/or menisci calcifications to diagnose radiographic CC.

US examination

All US examinations were performed by two physicians (one with five years of experience and the other with one year of experience). The operator was blinded to clinical, radiographic, and laboratory data. We used an HD15 US (Philips Ultrasound, Bothell, WA, USA)device equipped with a multi-frequency linear probe at a frequency of 5–12 MHz. US examinations were performed independently by a second sonographer, who was blinded to the findings of the first sonographer.

The US examination was performed according to the standardized techniques already described in literature for the identification of CPP deposits[6,14,15]. We evaluated CPP deposits in medial meniscus (MM), lateral meniscus (LM), HC, tendons (quadriceps and patellar tendons), and SF. To obtain the maximal exposure of the femoral cartilage surface, we obtained the transversal and longitudinal supra-patellar views with the knee in maximal flexion. MM and LM were assessed in flexion (30°) and complete extension. The knee tendons (quadriceps and patellar tendons) were examined with the joint in complete extension, semiflexion, and maximal flexion by transverse and longitudinal scans.

US image interpretation

The US features of CPPD were assessed according to the definitions of the OMERACT US task force[6,15] (Fig. 1). In case of disagreement between the sonographers, the results obtained by the more experienced sonographer were used to analyse the accuracy. The intra-observer reliability of 5-year experienced sonographer was calculated with static images obtained for all enrolled patients. The sonographer who was blinded to the clinical, previous US, and radiographic data reassessed these images at least 1 month after the initial US examination.

Statistical analysis

Statistical analyses were performed using the SPSS software package for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation or number (%). Student's *t* test was performed for analysing quantitative variables and Chi-squared test for categorical data. A *P* value less than 0.05 was considered statistically significant. We estimated the sensitivity, specificity, and positive and negative predictive values for US and radiography. The reference standard for the final diagnosis of CPPD was the presence of CPP crystals of SF. The area under receiver operating characteristic (ROC) curve was calculated. Comparisons of sensitivity and specificity between US and radiography were evaluated by the McNemar test. Inter- and intra-observer agreement were estimated by the kappa (κ) coefficient for dichotomous scoring. Kappa values of 0–0.20 were considered poor; 0.20–0.40, fair; 0.40–0.60, moderate; 0.60–0.80, good; and 0.80–1.00, excellent.

Results

Characteristics of the study population

We included 174 patients (105 females, 60.3%) with knee effusion. A total of 43 patients (24.7%) showed CPP crystals in SF. The remaining 131 control patients had osteoarthritis (OA) ($n = 67$), gout ($n = 26$), spondyloarthritis (SpA) ($n = 15$), rheumatoid arthritis (RA) ($n = 19$), Behcet's disease ($n = 2$), and systemic lupus erythematosus (SLE) ($n = 2$). Table I summarizes the demographic and clinical characteristics of study population. The clinical presentation of patients with CPPD in our study, according to the EULAR

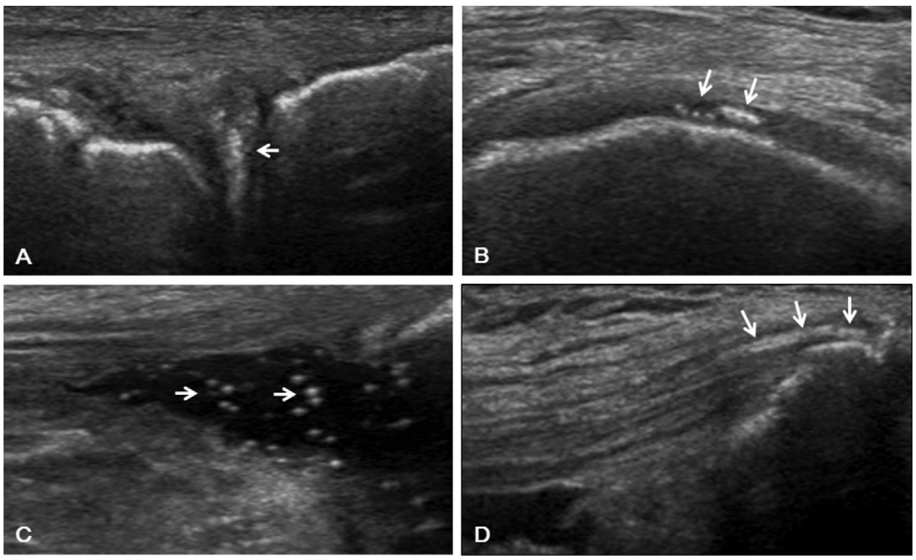

Fig. 1. Representative images showing knee ultrasound in CPPD disease. (A) Hyperechoic deposits within the lateral meniscus. (B) Hyperechoic deposits within the hyaline cartilage. (C) Hyperechoic deposits within the synovial fluid. (D) Hyperechoic deposits within the quadriceps tendon.

Table I
Demographic and clinical characteristics of study population. CPPD, calcium pyrophosphate deposition; CC, chondrocalcinosis; US, ultrasound; CPP, calcium pyrophosphate; MM, medial meniscus; LM, lateral meniscus; QT, quadriceps tendon; PT, patellar tendon

	CPPD ($n = 43$)	Controls ($n = 131$)	*P*-value
Age, mean (SD)	72.9 (15.5)	60.2 (15.6)	<0.001
Female, *n* (%)	30 (69.8)	75 (57.3)	0.156
Radiographic CC, *n* (%)	19 (44.1)	4 (3.1)	<0.001
US revealed CPP deposits			
Menisci (MM and/or LM), *n* (%)	28 (65.1)	24 (18.3)	<0.0001
Hyaline cartilage, *n* (%)	19 (44.2)	9 (6.8)	<0.0001
Tendon (QT and/or PT), *n* (%)	5 (11.6)	3 (2.3)	0.023
Synovial fluid, *n* (%)	15 (34.9)	29 (22.1)	0.095

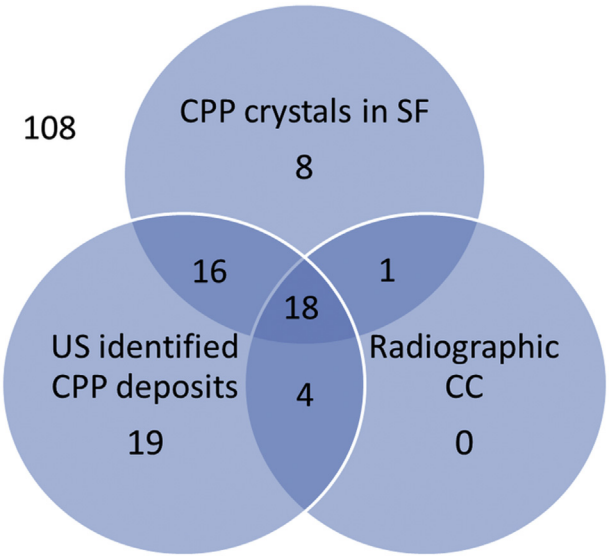

Fig. 2. Venn diagram identifying the distribution of CPP crystals in SF, US identified CPP deposits, and radiographic CC in all participants ($n = 174$). Among 131 patients in controls, 108 patients had neither US identified CPP deposits nor radiographic CC.

recommendations[4], was OA with CPPD in 25 patients (58.1%), acute CPP crystal arthritis in 16 patients (37.2%), and recurrent CPP crystal arthritis in two patients (4.7%). The mean symptom duration of CPPD was 3.2 ± 3.0 years.

US and radiographic assessment

US revealed CPP deposits in menisci (MM and/or LM), HC, and tendon (quadriceps and/or patellar tendon) more frequently in patients with CPPD than in control patients (Table I). The presence of CPP deposits in both MM and LM was significantly higher in the CPPD group than in the control group (62.6% and 60.4% vs 17.6% and 6.1%, $P < 0.0001$ and $P < 0.0001$, respectively). The presence of CPP deposits in quadriceps tendon was higher in the CPPD group than in the control group (9.4% vs 0.8%, $P = 0.014$), but the presence of CPP deposits in patellar tendon was not significantly different between the CPPD and control groups (4.6% vs 1.5%, $P = 0.256$). The presence of US CPP deposits in SF was not significantly different between two groups.

On CR of the knee joint, CC was more frequently observed in CPPD patients than in control patients ($P < 0.001$) (Table I). Figure 2 shows the distribution of CPP crystals in SF, US identified CPP deposits, and radiographic CC of study population.

Using the ROC curve, combined US evaluation of three components (menisci, HC, and tendon) showed better diagnostic accuracy compared with CR, combined US evaluation of four components (menisci, HC, tendon, and SF), and combined US evaluation of two components (menisci and HC) (Fig. 3). The area under ROC curve for US CPP deposits in menisci, HC, and tendon (excluding SF), and for radiographic CC was 0.758 (95% CI: 0.671–0.844) and 0.706 (95% CI: 0.604–0.080), respectively. Using SF analysis as the reference method, the sensitivity and specificity for US evaluation of three components (menisci, HC, and tendons) were 74.4% and 77.1%, and those for CR evaluation were 44.2% and 96.9%, respectively (Table II). US showed significantly higher sensitivity ($P = 0.001$), whereas CR was more specific ($P < 0.001$) in the identification of CPPD.

In the control group, US examination revealed CPP deposits in menisci, HC, SF, and tendon in 18.3%, 6.9%, 22.1%, and 2.3%, respectively. No difference in the prevalence of US identified CPP

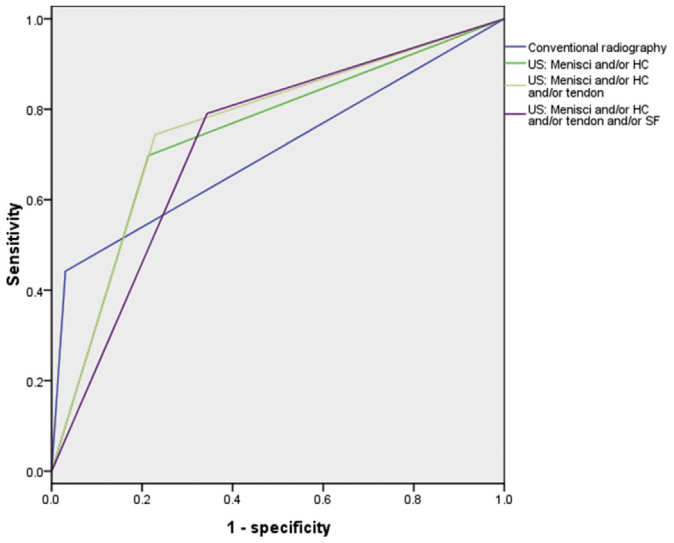

Fig. 3. Receiver operating characteristic curves for US and CR for detecting CPP deposits. The combined US evaluation of menisci, HC, and tendon produced the largest area under the curve [0.758 (95% CI: 0.671–0.844)].

deposits in menisci (MM and/or LM) ($P = 0.327$), HC ($P = 0.57$), SF ($P = 0.518$) and tendon ($P = 0.203$) was found among the three groups in controls (OA, gout, and other inflammatory arthritis including SpA, SLE, RA, and Behcet's disease). In the control group, positivity of Alizarin red S staining was higher in patients with US identified CPP deposits (51.5%) than those without US identified CPP deposits (26.7%) ($P = 0.006$). The proportion of patients with OA (44.8%) who had positive Alizarin red S staining results was significantly higher than that in patients with gout (15.4%) or other inflammatory arthritis (22.6%) ($P = 0.003$).

Agreement between US and conventional radiography

US revealed significantly more CPP deposits than CR ($P < 0.001$) at the knee joint level, independent from SF analysis. In all 174 patients, overall US identified CPP deposits (menisci and/or HC and/or tendon and/or SF) were observed in 79 knees, US CPP deposits except SF (menisci and/or HC and/or tendon) in 62 knees, and radiographic CC in 23 knees (Table III). The κ coefficient between overall US CPP deposits and radiographic CC was fair ($\kappa = 0.285$). US CPP deposits except SF (menisci and/or HC and/or tendon) and radiographic CC also showed fair agreement ($\kappa = 0.373$).

Inter- and intra-observer agreement

Inter-observer agreement was excellent for MM ($\kappa = 0.930$) and LM ($\kappa = 0.905$). The kappa values for HC ($\kappa = 0.844$) and SF ($\kappa = 0.817$) also showed excellent agreement. Inter-observer reliability for overall tendons (quadriceps and/or patellar tendons), quadriceps tendon, and patellar tendon showed the lowest agreement ($\kappa = 0.532$, 0.382, and 0.658, respectively). Intra-observer agreement was excellent for MM ($\kappa = 0.972$), LM ($\kappa = 0.942$), HC ($\kappa = 0.957$), quadriceps tendon ($\kappa = 0.828$), patellar tendon ($\kappa = 0.886$), and SF ($\kappa = 0.925$). Intra-observer reliability for overall tendon (quadriceps and/or patellar tendons) was good, but also showed the lowest agreement ($\kappa = 0.788$).

Table III
Knee ultrasound and radiography for CPPD at the joint level independent from SF analysis. All values are presented in numbers. CPPD, calcium pyrophosphate deposition; CPP, calcium pyrophosphate; SF, synovial fluid; US, ultrasound; CPP, calcium pyrophosphate; HC, hyaline cartilage

	Radiographic chondrocalcinosis		
	Presence	Absence	Total
Overall US CPP deposits[†]			
Presence	22	57	79
Absence	1	94	95
Total ($P < 0.0001$)[*]	23	151	174
US CPP deposits within menisci and/or HC and/or tendon			
Presence	21	41	62
Absence	2	110	112
Total ($P < 0.0001$)[*]	23	151	174

[*] $P < 0.0001$ based on Chi-squared test.
[†] Menisci and/or cartilage and/or tendon and/or SF.

Discussion

Although CPPD is one of the most common forms of inflammatory arthritis[2], CPPD tends to be underdiagnosed or misdiagnosed as gout and infectious arthritis. In one study, patients with OA who underwent knee replacement surgery were found to have 62% CPP crystals in their surgical specimen[9]. Therefore, in consideration of the increasing need for a sensitive and alternative test for diagnosis of CPPD, many studies have been performed to evaluate the diagnostic accuracy of US in CPPD[5]. However, the lack of a universally accepted definition and insufficient data for reliability make it difficult to confirm the diagnostic role of US in CPPD. Our study confirmed better diagnostic performance of US compared with CR and excellent US reliability at the level of menisci, HC, tendon, and SF of the knee, using the OMERACT definition for CPPD[6].

We assessed the US characteristics of CPPD at the level of menisci, HC, tendon, and SF of the knees using the new OMERACT definition. Previous studies have typically assessed CPP deposits in menisci and HC[10–12], whereas the present study also included CPP deposits in tendon and SF. The sensitivity and specificity of US in detecting CPPD vary depending on the structure under examination. In the present study, CPP deposits of the tendon showed low prevalence and sensitivity, but high specificity. This could be due to the late involvement of tendon structure in the natural course of CPPD[13]. On the other hand, the presence of CPP deposits in the SF did not contribute in distinguishing CPPD from other diseases, because the prevalence of CPP deposits in SF was not significantly different between the CPPD and control groups. Through

Table II
Performance of ultrasound and radiography of the knee for diagnosis of CPP deposition. Values are expressed as % (95% confidence interval). PPV, positive predictive value; NPV, negative predictive value; US, ultrasound; CPP, calcium pyrophosphate; HC, hyaline cartilage; SF, synovial fluid

	Sensitivity	Specificity	PPV	NPV	Accuracy
US CPP crystal deposition					
Menisci and/or HC	69.8 (53, 82)	78.6 (70, 85)	51.7 (38, 64)	88.8 (81, 93)	76.4 (70, 83)
Menisci and/or HC and/or tendon	74.4 (58,86)	77.1 (68, 83)	51.6 (38, 64)	90.2 (82, 94)	76.4 (70, 83)
Menisci and/or HC and/or tendon and/or SF	79.1 (63, 89)	65.6 (56, 73)	43.0 (32, 54)	90.5 (82, 95)	69.0 (62,76)
Radiographic chondrocalcinosis	44.2 (29, 56)	96.9 (91, 99)	82.6 (60, 94)	84.1 (77, 89)	83.9 (78, 89)

comparison of US-detected CPP deposits at different anatomical structure levels, we found that combined US evaluation of menisci, HC, and tendon showed the best diagnostic value. Our study results suggest that comprehensive US scanning of three components (menisci, HC, and tendon) can improve the diagnosis of CPPD. It is important to note, however, that only observing calcifications in SF could lead to overdiagnosis of CPPD.

In our study, the specificity (77.1%) and sensitivity (74.4%) of US were lower than those reported in previous studies, which ranged between 88–100% and 60–100%, respectively[10–12,14,16]. This could be explained by discrepancies in reference standards for CPPD and enrolled populations. Our study, similar to previously reported studies, used SF analysis as a reference standard for diagnosis of CPPD[4]. Although observing CPP crystals in the SF of the affected joint is the golden standard for identifying CPPD, one study demonstrated that SF analysis showed an absolute specificity (100%), but a low sensitivity (77.8%)[8]. Therefore, using the presence of CPP crystals in SF for diagnosis could not be a gold standard and have influenced the lower than expected diagnostic accuracy of US in the present study. And a key issue with compensated polarized light microscope is disparity in detecting crystals in physicians. Previous papers demonstrated unreliability of compensated polarized light microscope for CPP, and emphasized the need for improvement regarding crystal identification of particularly CPP and other non-MSU crystals[17]. In our study, 19 patients in control had US identified CPP deposits without radiographic CC and four patients in control had both US identified CPP deposits and radiographic CC. Possible explanations for these findings are low detection rate of CPP crystals in SF, misread of polarized light microscope for CPP crystals, false positive results of US and CR, and lower diagnostic value of US detected CPP deposits in SF than other structures (menisci, HC, and tendon). Further studies are needed to confirm the sensitivity and specificity of SF analysis for diagnosing CPPD, and to compare the diagnostic value with US evaluation.

In our study, 37.2% of patients in the CPPD group presented with acute CPP crystal arthritis. These patients could not achieve the maximal degree of knee flexion due to the large amount of effusion and pain. Thus, limited examination of femoral cartilage surface could have also influenced the low sensitivity in this study. Additionally, patients in the CPPD group in our study were relatively older than those in previous studies[8,12,14]. As our study might have included a high percentage of patients with end-stage OA[18], significant cartilage loss could be another reason for the lower sensitivity compared with other studies. In the CPPD group, the Kellgren–Lawrence grades were higher in patients without US CPP deposits in HC than in those with US CPP deposits in HC [median (interquartile range), 3 (1) vs 2 (1), $P = 0.048$] (data not shown). This also reflects the contribution of the loss and thinning of femoral cartilage in advanced OA to the low detection rate of CPP crystals in HC. Finally, the proportion of patients with OA in the control group was higher in the present study than in previous studies[11,12]. In our study, 44.8% of OA patients without CPP crystals showed positive Alizarin red S staining results. More patients with US identified CPP deposits showed positive results for this staining than patients without US identified CPP deposits in control group.

CPP and BCP are the two most common forms of calcium-containing crystals found in articular cartilage. CC is a term for cartilaginous calcifications, without detailing its chemical origin[19]. BCP crystals are known to contribute to the pathogenesis of OA and osteoclastogenesis[20]. BCP crystals are present in the SF of up to 60% of patients with OA, and they are present in the cartilage of up to 100% of affected joints at time of joint replacement surgery[21]. Although Alizarin red S staining is associated with a high false-positive rate[22], Alizarin red S staining is used to identify calcium-containing crystals such as BCP crystals. Therefore, our study results suggest that the calcifications detected using US could be not only CPP crystals, but also other calcium-containing crystals such as BCP. The OMERACT definitions for US features of CPPD are expert-opinion based, and the US identified CPP deposits could be a mixture of CPPD and BCP or composed solely of BCP deposits. The latter is especially likely to be true for observing calcifications in SF. This could influence the relatively low specificity of US in our study. Recently, advanced imaging techniques, including dual-energy computed tomography[23], advanced magnetic resonance imaging techniques, and diffraction-enhanced synchrotron imaging, have been used to detect CPP crystals[2]. One study showed that multi-energy spectral photon-counting CT could differentiate calcium hydroxyapatite from calcium oxalate[24]. However, to date, there have been no studies that conclusively prove that US detected hyperechoic lesion in tendon according to OMERACT definition is true CPP deposits. Further studies using new imaging technologies are needed to validate the OMERACT definition for CPPD and to distinguish various calcium crystals. Careful interpretation of US identified CPP deposits in the absence of SF analysis is needed in clinical practice.

Our study demonstrated excellent inter- and intra-observer agreement for CPP deposits at the level of menisci, HC, and SF of the knee. However, the tendon showed fair inter-observer and good intra-observer agreement. The relatively low agreement at the tendon might be related to the difficult applicability of the OMERACT definition, and reflect the need for a revised scanning method for tendon level assessment. Further studies are necessary to refine the definition of US characteristics of CPP deposits at the tendon level, to improve diagnostic accuracy and reliability among sonographers with different degrees of experience.

The present study has some limitations. First, it was a single-centre study, and the number of patients with CPPD was relatively small. Second, as per the STARD 2015 guidelines for reporting diagnostic accuracy studies, providing the exact number of participants who were excluded for the study using diagram helps readers to judge the risk of bias[25]. Although the estimated number of the population screened and excluded were 190 and 16, respectively, the exact number of patients at each stage of the study was not provided in our study. Third, our study was limited to evaluation of the knees. The wrist is the second most commonly involved joint[26,27], and previous studies showed a high specificity of US at the Achilles tendon and plantar fascia[13,28]. One study demonstrated that CC visualized on a CR commonly occurred at other joints such as hip, wrists, and symphysis pubis in the absence of knee CC[29]. Therefore, US examination for knee alone is an insufficient screening method for CPPD. Further studies are needed to assess the diagnostic accuracy of US in various CPP deposition sites. Fourth, our study used only Alizarin red S staining to detect BCP crystals. We speculated that calcifications detected using US could include not only CPP crystals but also other calcium-containing crystals such as BCP crystals. However, Alizarin red S staining is associated with a high false-positive rate[30]. Further studies, using more specific methods for BCP crystal detection, such as multi-energy CT[24], oxytetracycline staining, electron microscopy, and Raman spectroscopy[30], are needed to confirm our speculation. Finally, inter- and intra-observer agreement for SF analysis using a compensated polarizing microscope and CR were not evaluated, and we calculated intra-observer agreement with static images rather than with a twice-repeated US procedure. This may have also affected the intra-observer reliability.

In conclusion, US confirmed better diagnostic capacity than CR to diagnose CPPD, with excellent intra- and inter-observer reliability. Combined evaluation of menisci, HC, and tendon showed the best diagnostic performance at the knee level. Evaluating only hyperechoic deposits within the SF could lead to overdiagnosis of

CPPD. Considering the high sensitivity of US and the high specificity of CR, US and CR are complementary diagnostic examinations for CPPD.

Author contributions

All authors made substantial contributions to the conception and design of the study, data analysis and interpretation, drafting the article or critical revision for important intellectual content, and final approval of the submitted version.

Competing interest statement

The authors declare that they have no completing interest.

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