APPENDIX

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DIAGNOSIS OF CALCIUM PYROPHOSPHATE CRYSTAL DEPOSITION DISEASE BY ULTRASOUND: WHICH SITES SHOULD BE SCANNED?

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1. Background

The gold standard for the diagnosis of calcium pyrophosphate deposition disease (CPPD) is the identification of calcium pyrophosphate (CPP) crystals in the synovial fluid by compensated polarized light microscopy, or occasionally, in biopsied tissues [1]. However, synovial fluid aspiration and/or analysis may not be always feasible for several reasons, especially in primary care or emergency medicine settings where synovial fluid analysis is rarely performed [2]. Therefore, imaging techniques have gained a central role in the diagnosis of CPPD [3]. In particular, ultrasonography (US) has emerged as one of the most valid, accurate and reliable tools for the diagnosis of CPPD in daily practice, having shown a greater sensitivity and comparable specificity to conventional radiography (CR) in the detection of CPP crystal aggregates [4]. Nevertheless, according to a recent survey carried out by the Gout, Hyperuricemia and Crystal-Associated Disease Network (G-CAN) Experts, the development of internationally accepted protocols, standards and consensus for US imaging in CPPD remains one of the most important research priorities [5]. In fact, previous US studies have separately analyzed the diagnostic value of US in the assessment of different tissues and joints in comparison with different reference standards [6], and, to date, no studies have investigated the optimal US scanning protocol in the diagnosis of CPPD.

2. Objectives

To identify the most effective US scanning protocol in the diagnosis of CPPD in inter-critical periods by a) assessing the diagnostic value of the combinations of OMERACT-defined US findings of CPP deposits in the upper and lower limbs and b) selecting the best minimal combination of anatomic structures to be scanned.

3. Overall study design

This is a multi-centre cross-sectional study in which patients with a crystal-proven diagnosis of CPPD and disease-controls with other rheumatic diseases will be consecutively recruited.

4. Methods

4.1 Population of interest

The inclusion criteria for CPPD patients are:

- Crystal proven-diagnosis of CPPD
- Aged older than 18
- Able to provide informed consent, according to requirements of local IRB/ethics committee

The exclusion criteria for CPPD patients are:

- Known history of other inflammatory arthropathies
- Joint injections in the last 3 months
- Previous major trauma, fracture or surgery of the joints included in the scanning protocol.

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The inclusion criteria for disease controls are:

- A diagnosis of rheumatic disease (other than CPPD) according to international classification criteria
- One or more synovial fluid analyses negative for CPP crystals
- Aged older than 18
- Able to provide informed consent, according to requirements of local IRB/ethics committee

The exclusion criteria for disease controls are:

- Joint injections in the last 3 months
- Previous major trauma, fracture or surgery of the joints included in the scanning protocol.

4.2 Evaluation schedule

Each centre can enroll consecutive patients who will be seen for routine or urgent CPPD care following local scheduling processes, without applying any further selection criteria, and with all sites enrolling selected age- and sex- matched disease-controls with other rheumatic diseases.

Clinical assessment

All patients with CPPD and disease-controls will undergo a standard clinical assessment. The following clinical and laboratory data will be registered in all the patients: age, sex, body mass index (BMI) and disease duration since the diagnosis. In addition, CPPD etiology (i.e. idiopathic, familiar or associated with metabolic conditions) and clinical presentation according to EULAR recommendations [1] were recorded in patients with CPPD.

Sonographic assessment

On the same day, one or more sonographers in each centre will carry out all the US examinations, blinded to clinical data. The gray-scale setting parameters will be adapted to enhance the CPP crystals recognition. CPP deposits will be defined according to the Outcome Measure in Rheumatology (OMERACT) definitions [7] and scored in a binary fashion.

Each patient with CPPD and each disease-control will undergo a systematic and multiplanar US examination of 18 hyaline cartilages, 12 fibrocartilages, 10 tendons, 2 joint recesses and 2 ligaments as follows: shoulder (glenoid fibrocartilage, humeral hyaline cartilage and acromioclavicular fibrocartilage), elbow (humeral hyaline cartilage and triceps tendon), wrist (triangular fibrocartilage, scapho-lunate ligament, volar recess of the radio-lunate joint), hand (hyaline cartilage of the metacarpophalangeal joints from 2nd to 5th finger), hip (acetabular fibrocartilage and femoral hyaline cartilage), knee (femoral condyles' hyaline cartilage, meniscal fibrocartilages, patellar and quadriceps tendons), ankle (talar hyaline cartilage and Achilles tendon), foot (plantar fascia) (TABLE 1).

US examinations will be conducted according to the 2017 EULAR standardised procedures for US imaging in rheumatology [8].

Table 1. Scanning protocol		
Anatomic Site	Anatomic Target	EULAR Standard Scan [8]
Shoulder	Glenoid fibrocartilage	S21
	Humeral hyaline cartilage	S21, S22
	Acromioclavicular fibrocartilage	S05
Elbow	Humeral hyaline cartilage	E01, E02
	Triceps tendon	E15, E16
Wrist	Triangular fibrocartilage	W10, W11
	Dorsal component of the scapho-lunate ligament	W07, W08
	Volar recess of the radio-lunate joint	W29, W39, W40
Hand	Hyaline cartilage of the metacarpophalangeal joints from 2^{nd} to 5^{th} digit	W19
Hip	Acetabular fibrocartilage	H1, H2, H4
	Femoral hyaline cartilage	H1, H2, H3
Knee	Femoral condyles' hyaline cartilage	K20, K21
	Medial meniscus fibrocartilage	K06
	Lateral meniscus fibrocartilage	K08
	Quadriceps tendon	K2, K3, K5
	Patellar tendon	K11, K12, K14, K16
Ankle	Talar hyaline cartilage	A02
	Achilles tendon	A47, A49, A51
Foot	Plantar fascia	A52

Inter and intra-observer reliability

Inter-observer and intra-observer reliability of the US assessment has been evaluated in a previous OMERACT exercise using the OMERACT methodology.

4.3 Analysis plan

Statistical analysis will be performed using the Statistical Package for Social Sciences, v.26 (Chicago, Il, USA). Quantitative variables will be reported as the mean±standard deviation (SD). Qualitative variables will be presented as absolute frequency and/or corresponding percentage. Quantitative variables will be compared

using the Student t test or Mann–Whitney test, as appropriate. Qualitative variables will be compared using the Fisher's exact test (2×2 tables).

To select the optimal scanning protocols in the diagnosis of CPPD we will investigate which US findings and anatomic sites will be more frequently involved in patients with CPPD, as a first step. These results will be reported using a "prevalence and distribution" table.

In the second step, we will calculate separately the diagnostic accuracy for each US finding and each anatomic site obtained in the first step. These results will be reported describing the sensitivity, specificity, positive predictive value and negative predictive value of each US finding in each anatomic site (e.g. hyaline cartilage calcification of the knee, hyaline cartilage calcification of the hip, fibrocartilage calcification of the knee, fibrocartilage calcification of the hip).

In the third step, we will define the minimal combination of US-detected abnormalities and anatomic sites yielding the best diagnostic performances by combining each US finding in each anatomic site. We will use an adaptive LASSO technique. The diagnostic performance of each combination will be compared using ROC curves. In case of a non-significant difference, the easiest scanning will be selected.

Diagnostic accuracy of US findings will be expressed as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Each measure will be presented with its 95% confidence interval. We expected a sensitivity of US of 90% and a specificity of US of 92% considering an expected error of 5% in each parameter.

Inter- and intra-observer reliability will be measured by unweighted Cohen's kappa and interpreted according to Landis and Koch (0–0.20 poor, 0.20–0.40 fair, 0.40–0.60 moderate, 0.60–0.80 good and 0.80–1 excellent).

Patients with missing data will be excluded from the analyses.

A p value <0.05 was considered significant.

4.4 Sample size

Sample size will be calculated using the following data:

- alpha error: 5%
- estimation error (d): 7%
- sensitivity of US in the diagnosis of CPPD: 85% [9]
- specificity of US in the diagnosis of CPPD: 85% [9]
- prevalence rate of CPPD in our cohort: 50%, assuming an enrollment ratio of 1:1

To obtain an estimated sensitivity of 85%, a total of 200 patients (100 CPPD patients and 100 disease controls) would be required. Similarly, to obtain an estimated specificity of 85%, a total of 200 patients (100 CPPD patients and 100 disease controls) would be required. Thus, the total required sample size would be 200 (100 CPPD patients and 100 disease controls).

5.1 Authorship

For each site recruiting $\geq$ 30 participants, two researchers will be identified for inclusion as authors on the publications generated from this project. For each additional 30 participants recruited at each site, a further author from each site can be included, if requested. In addition, those who contributed to the protocol development will also be included on the author list.

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5.2 Funding

This research will not receive specific grant from any funding agency in the public, commercial, or not-for-profit sectors. Patients will not receive any payment for the participation in the study.

5.3 ETHICS

This protocol and the template informed consent forms will be reviewed and approved by the Ethics Committee of the Coordinator Centre, named "CERM - Comitato Etico Regione Marche" and other local Ethics Committees (ECs) with respect to scientific content and compliance with applicable research and human subjects' regulations.

Each important modification to the protocol (e.g., changes to eligibility criteria, outcomes analyses) or to its relevant parties (e.g., investigators, HRECs, trial participants, trial registries, journals, regulators) will be submitted to the CERM and local ECs.

Each member of the study will obtain informed consent from every potential participant for appropriate consent procedures for persons with at age ≥18. Informed consent is required for the participation. Anonymization of gathered data will be guaranteed to each subject.

Given the fact that the study is observational and US has a very high safety profile, no insurance is required for this study.

5.4 DISSEMINATION PLAN

The results of this study will be submitted for publication to peer-reviewed journals or for presentation at national and international congresses. Each paper or abstract, must be reviewed and approved by the executive committee prior to submission.

Public access to the full protocol, participant-level dataset, and statistical code will be granted to all the researchers upon reasonable request.

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Supplementary Table S1. Demographic and clinical data of patients with CPPD disease included in study [10]

Age, years (mean±SD)	73.3±11.5
Sex, female (n, %)	54 (64.3%)
Body mass index, kg/m^2 (mean±SD)	24.9±3.2
Presence of CPP crystal at the SF analysis (n, %)	84 (100%)
Duration since the diagnosis (median and IQR))	2.5 (0.5-3.0)
CPPD disease aetiology	
- Idiopathic (n, %)	79 (94.0%)
- Associated with predisposing conditions (n, %)	5 (6.0%)
EULAR CPPD disease clinical presentation	
- Osteoarthritis + CPPD (n, %)	39 (46.4%)
- Acute CPP crystal arthritis (n, %)	39 (46.4%)
- Chronic CPP crystal inflammatory arthritis (n, %)	6 (7.1%)

CPP: calcium pyrophosphate, **CPPD**: calcium pyrophosphate deposition, **IQR**: interquartile range, **SD**: standard deviation, **SF**: synovial fluid.

Supplementary Table S2. Sonographic scanning protocols for the evaluation of the DC sign.

	Authors	Cipolletta et al.	Cipolletta et al.	Cipolletta et al.
	Year of publication	2021	2022	2022
	Reference	[9]	[10]	[11]
HCs included in the scanning protocol	HC of the femoral head at the hip	N	N	Y
	HC of the femoral condyles at the knee	Y	Y	Y
	HC of the talus at the ankle	Y	Y	Y
	HC of the 1st metatarsal head	Y	Y	N
	HC of the humeral head at the shoulder	N	N	Y
	HC of the radial capitellum and humeral trochlea at the elbow	N	N	Y
	HC of the radiocarpal and intercarpal joints at the wrist	Y	Y	N
	HC of the 2nd metacarpal head	Y	Y	Y

DC: double contour, **HC**: hyaline cartilage, **N**: no, **Y**: yes.

Supplementary Video S1

The video shows the typical aspect of the DC sign at the 1st metatarsophalangeal joint in a patient with gout. The abnormal hyperechoic band (the DC sign) moves together with the hyaline cartilage of the metacarpal head during the joint movement. In such a case, crystal deposits lie on the surface of the hyaline cartilage. Legend. m: metacarpal bone, p: proximal phalanx, arrows: DC sign.

Supplementary Video S2

The video shows the typical aspect of the pseudo DC at the knee joint in a patient with CPPD. The abnormal hyperechoic band moves in the opposite direction to the hyaline cartilage of the femoral condyle during the joint movement. In such a case, crystal deposits are located on the joint capsule of the suprapatellar recess. Legend. f: femur, p: patellar, arrows: pseudo DC sign.

Supplementary Video S3

The video shows the aspect of the cartilage interface sign at the knee joint in a healthy subject. The outer margin of the hyaline cartilage appears as a thin, hyperechoic line visible where the US beams perpendicularly insonate the hyaline cartilage. Legend. f: femur, arrow: cartilage interface sign.