Persistence of Crystals in Stored Synovial Fluid Samples

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ABSTRACT. ***Objective.*** Lack of access to polarized light microscopy is often cited as an argument to justify the clinical diagnosis of crystal-related arthritis. We assessed the influence of time since sampling and preservation methods on crystal identification in synovial fluid (SF) samples under polarized light microscopy.

Methods. This was a prospective, longitudinal, observational factorial study, analyzing 30 SF samples: 12 with monosodium urate (MSU) crystals and 18 with calcium pyrophosphate (CPP) crystals. Each SF sample was divided into 4 subsamples (120 subsamples in total). Two were stored in each type of preserving agent, heparin or ethylenediamine tetraacetic acid (EDTA), at room temperature or at 4°C. Samples were analyzed the following day (T1), at 3 days (T2), and at 7 days (T3) by simple polarized light microscopy, and the presence of crystals was recorded.

Results. The identification of crystals in the MSU group was similar between groups, with crystals observed in 11/12 (91.7%) room temperature samples and in 12/12 (100%) refrigerated samples at T3. Identification of CPP crystals tended to decrease in all conditions, especially when preserved with EDTA at room temperature [12/18 (66.7%) at T3], while less reduction was seen in refrigerated heparin-containing tubes.

Conclusion. Preserving samples with heparin in refrigerated conditions allows delayed microscopic examination for crystals. Avoiding crystal-proven diagnosis because of the immediate unavailability of microscopy no longer appears justified. (J Rheumatol First Release August 1 2020; doi:10.3899/jrheum.190468)

Key Indexing Terms:
CRYSTAL-RELATED ARTHRITIS
CALCIUM PYROPHOSPHATE CRYSTALS
MONOSODIUM URATE CRYSTALS
SYNOVIAL FLUID STORAGE

Synovial fluid (SF) analysis under polarized microscopy is an immediate, reliable, reproducible procedure requiring no staining or fixation that enables a definitive diagnosis of crystal-related arthritis[1,2]. The technique is included in the American College of Rheumatology Core Curriculum for specialty training[3]. Microcrystals that usually trigger arthritis, monosodium urate (MSU) and calcium pyrophosphate (CPP), are identified based on their shape and birefringence. Formally, a compensated polarized light microscope is required, although access can be limited. MSU crystals are recognizable by their needle shape and strong birefringence, while CPP crystals appear as parallelepipeds (rhomboidal, rods) with varying intensity of birefringence, often lower than that of MSU[4,5,6].

The time elapsed from joint aspiration to microscopic evaluation is a relevant consideration in SF analysis for crystals. Early visualization is usually recommended to prevent leukocyte degradation and potential crystal alteration, and to reach a rapid diagnosis[4]. However, only a few studies have set out to establish the correct timing and sample preservation methods for SF analysis.

Table 1 describes studies on SF analysis from our literature review[7,8,9,10,11,12,13]. Results differed significantly between studies, reflecting differences in the type of crystal assessed, methods, storage temperature, and timing. This heterogeneity makes direct comparison difficult and precludes a clear conclusion. Thus, the influence of the time between SF extraction and examination, and the preservation method, on the persistence of crystal visualization under an optical microscope remains to be determined. Our objective was to assess the persistence of crystals, MSU or CPP, over time

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Accepted for publication January 15, 2020.

Table 1. Published studies evaluating the storage of synovial fluid samples for crystal analysis.

Study	No. Samples	Crystals Analyzed	Preservation Method	Storage Temperature	Time Period	Conclusions	
Bible[7]	50	MSU	NA	Refrigerated (temperature NA) vs room temperature	24 h	Crystals were visible at 24 h	No differences at different storage temperatures
Kerolus[8]	50 (5 CPP, 7 MSU, 8 lipids, 6 apatite)*	CPP, MSU, others (lipids, apatite, hematoidin)	Heparin	4°C and 22°C	2 mo	CPP shows dissolution over time, while MSU crystals persist but in smaller number	Emergence of artifacts
McKnight[9]	6	CPP	Unclear (EDTA, heparin, saline, and no anticoagulant for samples from the same patient)	Room temperature	4 wks	CPP persistence at room temperature	
McGill[10]	11	CPP	No anticoagulant, some with heparin	Room temperature, 4°C, and –70°C	8 wks	Persistence with a slight decrease in crystal counts at room temperature and 4°C	No new crystals
Galvez[11]	91 (31 MSU, 30 CPP, 30 no crystals)	MSU, CPP, no crystals	Heparin, EDTA, no anticoagulant	4°C (plus –80°C with no anticoagulant)	24 h vs 72 h 2 mos later, at –80°C	High probability of detecting crystals if those were present first**	EDTA associated with less agglomeration and better cellular delineation
Tausche[12]	75 (16 MSU, 6 CPP, 5 MSU + CPP, 48 no crystals)	MSU, CPP	EDTA or no anticoagulant	20°C and 4°C	3 days	No changes in crystal counts for both types, regardless of storage conditions	No new crystals
Kienhorst[13]	10	MSU	No anticoagulant	–20°C, 4°C, and 20°C	24 wks	Persistence in identification of crystals with no differences between storage temperature	
Present work	30 (12 MSU, 18 CPP)	MSU, CPP	EDTA or heparin	20°C and 4°C	7 days	Persistence of MSU regardless of storage conditions	Decrease of CPP visualization at room temperature and with EDTA preservation

*Each type of crystal is analyzed independently, and there may be samples without crystals or with several types; thus, the total number of samples is not consistent. **In samples at –80°C visualized after 2 months, the observed decrease in intracellular CPP could be explained by the cell degradation over time. EDTA: ethylenediamine tetraacetic acid; NA: not available; CPP: calcium pyrophosphate; MSU: monosodium urate.

in SF following extraction, according to the sample method and storage temperature.

MATERIALS AND METHODS

This was a prospective, longitudinal, observational factorial study. SF samples were obtained from clinical practice at the Rheumatology Section of the Hospital General Universitario de Alicante, Alicante, Spain. No clinical data were collected, making ethical evaluation unnecessary according to current regulations in Spain.

Sample collection. The sampling period was from October 2017 to April 2018. Inclusion criteria were that SF samples showed MSU or CPP crystals under polarized light microscopy, and that they were identified by a rheumatologist with expertise in the field (MA). Samples containing both types of crystals were excluded.

The SF was divided into 4 subsamples. Two were stored in tubes containing heparin or ethylenediamine tetraacetic acid (EDTA) as preserving agents, at either room temperature (20°C) or in refrigeration at 4°C. In all cases, the tubes were protected from light and numbered in a nonconsecutive manner. The list of numbers was kept separately, and the study started when 2 SF samples were available on the same day. To prevent bias, 3 control fluid samples without crystals were also included.

Samples were analyzed using a simple polarized light microscope (Olympus model CX41) at 400× magnification under bright and simple (noncompensated) polarized light. Crystals were classified based on their shape and intensity of birefringence[4]. Neither other magnifications nor red compensator were used. Samples were labeled as lacking crystals after examination of a minimum of 30 separated 400× fields on the slide. Observations were performed the following day (T1), at 3 days (T2), and at 7 days (T3). The observer was a final-year medical student who received specific training prior to the study, and the technique was reviewed after every 10 samples analyzed[14]. Observations were recorded and submitted separately for each timepoint, and they could not be consulted again until the end of the study.

The primary outcome variable was the presence of crystals. Secondary outcome variables were the type of crystal (MSU or CPP) and their predominant location (intracellular, extracellular, inside conglomerates, or various, depending on where the principal location could be established). Explanatory variables were the time elapsed between sample collection and analysis (T1, T2, and T3), the preserving agent (EDTA or heparin), and the storage temperature (20°C or 4°C).

Statistical analysis. Descriptive data were expressed as means (± SD) for quantitative variables, and as absolute and relative frequencies for qualitative variables. To assess the influence of variables in the identification of crystals, a generalized linear model for repeated measures was built, with fixed effects (type of crystal; temperature; preserving agent) and random effects (sample observations with the time). Presence of crystals was considered the dependent variable. In case of significant results, interactions between explanatory variables were assessed accordingly. Statistical analyses were performed using Microsoft Excel and Google Colab with Jupyter notebooks, libraries Pyreadstat v0.2.0, Pandas v0.23.3, and Statsmodels v0.10.1. The level of significance was established at $p < 0.050$.

Quality of data assessment. The Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool[15,16] was used to evaluate the quality of the studies identified from the literature search and the present study.

This tool assesses risk of bias according to the following domains: patient selection; index test (storage temperature, conservation and time); reference standard (crystals at baseline); and flow and timing. Except for the last domain, applicability was assessed as well. Two authors (SP and JAB) independently applied the QUADAS-2 tool, resolving disagreements by consensus.

RESULTS

Thirty SF samples with crystals (MSU in 12, CPP in 18) were divided into 120 subsamples at baseline, so 360 observations were carried out at T1, T2, and T3. The observer correctly identified the 3 fluids without crystals, and these were excluded from the analysis.

Microscopic examinations were performed at the different timepoints as follows: T1, mean 31.0 h (SD 10.3); T2, mean 90.5 h (SD 29.3); and T3, mean 179.2 h (SD 16.4). By type of crystal, the mean time between sampling and analysis was, for MSU: T1, 31.2 h (SD 11.8); T2, 99.2 h (SD 34.9); and T3, 182.9 h (SD 14.8). For CPP, mean time elapsed was T1, 30.8 h (SD 9.2); T2, 84.7 h (SD 23.3); and T3, 176.8 h (SD 17.1).

Table 2 and Figure 1 show the results of the microscopy observations for each type of crystal (presence and location) at each timepoint, in relation to the preservative used and storage temperature, as well as the results of the statistical comparisons. MSU crystals seemed to persist in almost all samples, regardless of temperature and preserving agent

Table 2. Results of sample analysis for crystals at each timepoint, by type of crystal, preserving agent, and storage temperature. Data are shown as % (number).

Crystal	Temperature	Preserving Agent	T0	T1		T2		T3	
			Presence	Presence	Location	Presence	Location	Presence	Location
MSU	20°C	EDTA	100 (12)	91.7 (11)	None: 8.3 (1) IC: 16.7 (2) EC: 33.3 (4) CO: 0 (0) V: 41.7 (5)	91.7 (11)	None: 8.3 (1) IC: 0 (0) EC: 33.3 (4) CO: 0 (0) V: 58.3 (7)	91.7 (11)	None: 8.3 (1) IC: 0 (0) EC: 33.3 (4) CO: 0 (0) V: 58.3 (7)
		Heparin	100 (12)	91.7 (11)	None: 8.3 (1) IC: 16.7 (2) EC: 33.3 (4) CO: 0 (0) V: 41.7 (5)	91.7 (11)	None: 8.3 (1) IC: 0 (0) EC: 33.3 (4) CO: 0 (0) V: 58.3 (7)	91.7 (11)	None: 8.3 (1) IC: 0 (0) EC: 16.7 (2) CO: 8.3 (1) V: 66.7 (8)
	4°C	EDTA	100 (12)	100 (12)	None: 0 (0) IC: 8.3 (1) EC: 41.7 (5) CO: 0 (0) V: 50.0 (6)	100 (12)	None: 0 (0) IC: 0 (0) EC: 25.0 (3) CO: 8.3 (1) V: 66.7 (8)	100 (12)	None: 0 (0) IC: 0 (0) EC: 25 (3) CO: 8.3 (1) V: 66.7 (8)
		Heparin	100 (12)	91.7 (11)	None: 8.3 (1) IC: 8.3 (1) EC: 33.3 (4) CO: 0 (0) V: 50.0 (6)	100 (12)	None: 0 (0) IC: 0 (0) EC: 41.7 (5) CO: 0 (0) V: 58.3 (7)	91.7 (11)	None: 8.3 (1) IC: 0 (0) EC: 25.0 (3) CO: 0 (0) V: 66.7 (8)
CPP	20°C	EDTA	100 (18)	88.9 (16)	None: 11.1 (2) IC: 66.7 (12) EC: 5.6 (1) CO: 0 (0) V: 16.7 (3)	77.8 (14)	None: 22.2 (4) IC: 55.6 (10) EC: 0 (0) CO: 0 (0) V: 22.2 (4)	66.7 (12)	None: 33.3 (6) IC: 55.6 (10) EC: 0 (0) CO: 0 (0) V: 11.1 (2)
		Heparin	100 (18)	100 (18)	None: 0 (0) IC: 55.6 (10) EC: 5.6 (1) CO: 11.1 (2) V: 27.8 (5)	83.3 (15)	None: 16.7 (3) IC: 44.4 (8) EC: 0 (0) CO: 5.6 (1) V: 33.3 (6)	77.8 (14)	None: 22.2 (4) IC: 16.7 (3) EC: 0 (0) CO: 16.7 (3) V: 44.4 (8)
	4°C	EDTA	100 (18)	83.3 (15)	None: 16.7 (3) IC: 77.8 (14) EC: 0 (0) CO: 0 (0) V: 5.6 (1)	94.4 (17)	None: 5.6 (1) IC: 61.1 (11) EC: 0 (0) CO: 0 (0) V: 33.3 (6)	77.8 (14)	None: 22.2 (4) IC: 38.9 (7) EC: 0 (0) CO: 11.1 (2) V: 27.8 (5)
		Heparin	100 (18)	88.9 (16)	None: 11.1 (2) IC: 27.8 (5) EC: 0 (0) CO: 5.6 (1) V: 55.6 (10)	100 (18)	None: 0 (0) IC: 22.2 (4) EC: 0 (0) CO: 27.8 (5) V: 50.0 (9)	83.3 (15)	None: 16.7 (3) IC: 16.7 (3) EC: 0 (0) CO: 16.7 (3) V: 50.0 (9)

CO: conglomerates; CPP: calcium pyrophosphate; EC: extracellular; IC: intracellular; MSU: monosodium urate; V: various; EDTA: ethylenediamine tetraacetic acid; T0: baseline; T1: following day; T2: 3 days after storage; T3: 7 days after storage.

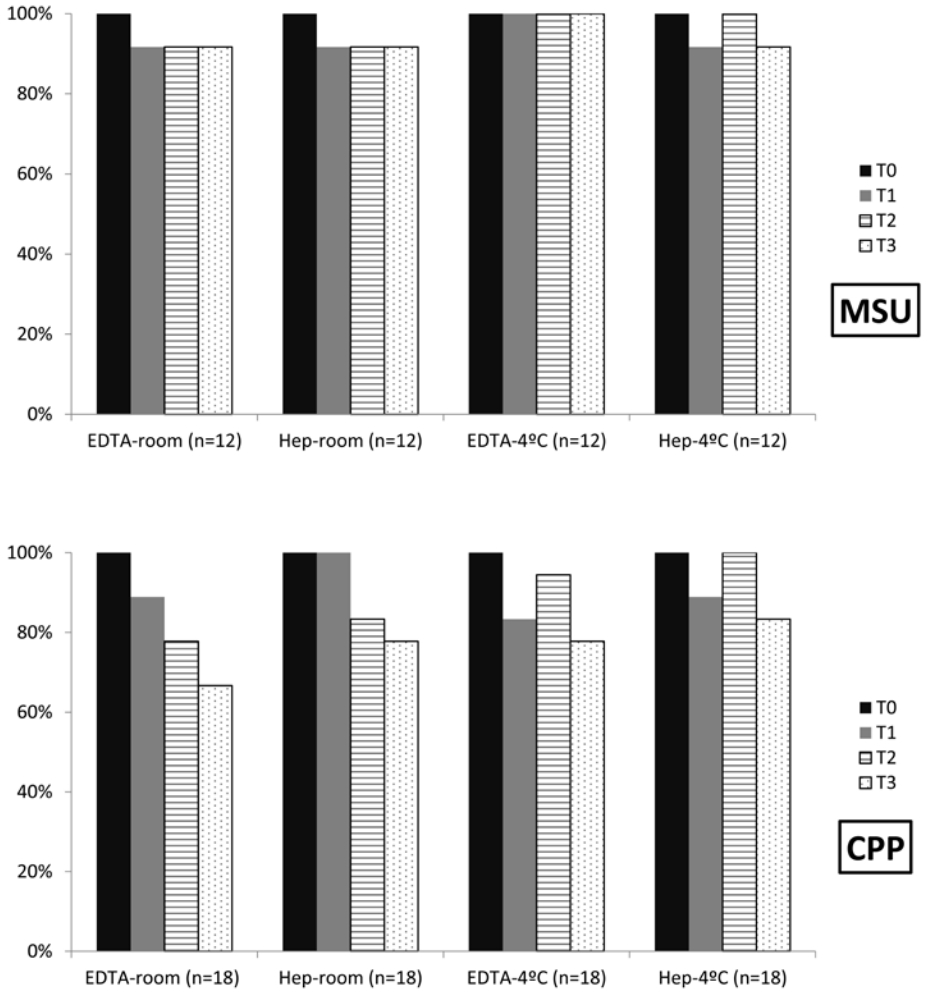

Figure 1. Persistence of monosodium urate crystals (MSU; top) and calcium pyrophosphate crystals (CPP; bottom) at each timepoint, according to preservative and storage temperature. EDTA: ethylenediamine tetraacetic acid; HEP: heparin.

(Figure 1). However, the persistence of CPP crystals tended to decline progressively in all samples, especially when kept in EDTA and stored at room temperature, with only 60% showing crystals by microscopy.

The results of the generalized linear model confirmed the descriptive results (Table 3). Time to visualization showed a significant, inverse association with crystal identification in the stored samples. As well, in comparison to CPP, MSU crystals were positively associated with the identification. No association with temperature or preserving agents was found. Then the analysis was stratified for the type of crystal (Table 3). For MSU crystal, no explanatory variable was found associated with the visualization, in keeping with our descriptive results of crystal persistence regardless of time, temperature, or preserving agent. However, for CPP crystals, time showed a significant, inverse association, while refrigerated samples showed a direct association. Interestingly, a significant interaction between both variables was noted. No association was found for the type of preserving agent used.

The Supplementary Table 1 (available with the online version of this article) shows the results of the association analysis incorporating only the T1 to T3 observations.

DISCUSSION

SF analysis under polarized light microscopy remains the reference standard for diagnosing crystal-related arthritis[1,2], and it is an essential procedure when confronting arthritis of unknown origin[17]. However, clinicians sometimes avoid performing it in practice[18,19], citing the lack of immediate access to a microscope as justification. Our results support the identification of crystals in SF, especially in the case of MSU, up to 1 week after sampling, regardless of the storage temperature or the preserving agent. Regarding CPP crystals, their identification decreased over time, especially when samples were kept at room temperature and preserved using EDTA. However, samples that were refrigerated and

Table 3. Results of the association analysis by generalized linear models, with presence of crystals as the dependent variable.

Sample	Variable	Coefficient (95% CI)	p
Whole	Time	–0.0013 (–0.001 to –0.002)	< 0.001
	Type of crystals		
	CPP	Ref	—
	MSU	0.0603 (0.011 to 0.110)	0.018
	Temperature		
	Room	Ref	—
	Refrigerated	0.0391 (–0.009 to 0.088)	0.114
	Preserving agent		
	EDTA	Ref	—
	Heparin	0.0237 (–0.025 to 0.072)	0.338
MSU crystals	Time	0.0007 (–0.000 to 0.002)	0.170
	Temperature		
	Room	Ref	—
	Refrigerated	0.0402 (–0.032 to 0.113)	0.277
	Preserving agent		
	EDTA	Ref	—
	Heparin	0.0548 (–0.018 to 0.127)	0.138
CPP crystals	Time	–0.0026 (–0.001 to –0.004)	< 0.001
	Temperature		
	Room	Ref	—
	Refrigerated	0.1715 (0.062 to 0.281)	0.002
	Preserving agent		
	EDTA	Ref	—
	Heparin	–0.0851 (–0.195 to 0.024)	0.128
	Time*temperature	–0.0021 (–0.004 to –0.001)	0.006
	Time*preserving	0.0010 (–0.001 to 0.002)	0.206

EDTA: ethylenediamine tetraacetic acid; CPP: calcium pyrophosphate; MSU: monosodium urate.

preserved in heparin showed excellent persistence of CPP crystals at 3 days (100%) and good persistence at 7 days (83.3%). Thus, microscope analysis can be reliable for up to several days after the sample is taken owing to crystal persistence and the apparent absence of *de novo* formation. Regarding the findings on CPP crystals, samples can be refrigerated in a heparinized medium (common tubes for biochemical tests) to ensure the persistence of crystals when they are present.

Compared to reports in the literature (Table 1), our findings are in keeping with those of Gálvez, *et al*[11] and Tausche, *et al*[12], except for the gradual reduction in identification of CPP crystals, linked to room temperature and EDTA preservation. These differences may be attributable to several factors. First, previous studies used a time frame of 3 days, while our samples were stored for up to 7 days, allowing more time for the degradation of crystals (comparisons from baseline to T2 showed no significant differences). Second, unlike other studies, ours maintained strict blinding during the examination of samples. Besides numerically labeling the study samples, the results of each observation were sealed, impeding comparisons with previous observations, and fluids with no crystals were introduced as controls. These measures helped to reduce risk of bias, strengthening the certainty of the evidence. Table 4 shows the quality assessment of the published studies along with a self-assessment of this one. Most were deemed to provide low- or moderate-quality evidence, except for Gálvez, *et al*[11], Tausche, *et al*[12], and our study, which were at low risk of bias.

MSU crystals remained identifiable throughout the 7-day study period, independent of the storage conditions. Artificial MSU crystals can be synthesized using oversaturated concentrations of urate and sodium. However, this solution may remain metastable for long periods until crystallization occurs. Factors such as albumin enhance MSU nucleation, while alkaline pH delays it[20]. After formation, solubility of MSU crystals mostly and directly depends on temperature[20,21,22,23,24]. Here, tubes were kept at lower temperatures (4°C and 20°C), likely reducing dissolution of crystals and contributing to their persistence in stored samples. Storage of tubes at normal body temperature (36°C) may be of further research interest to assess MSU crystal degradation. Theoretically, crystals could form continuously in stored samples, because SF urate levels tend to be higher than serum levels in patients with untreated gout, widely exceeding the saturation point for urate[25]. However, while *de novo* crystallization of MSU *in vitro* may occur in sealed glass slides, it is rare in stored samples[24]. Besides, this is not in keeping with the current understanding of how

Table 4. QUADAS-2 quality assessment of diagnostic accuracy test studies evaluating the storage of synovial fluid samples. Unclear risk is shown as "?"[15].

Study	Domain	Patient Selection	Index Test	Reference Standard (crystals at baseline)	Flow and Timing
Bible[7]	Risk of bias	Low risk	Storage temperature: High risk Conservation: High risk Time: Low risk	?	Low risk
	Applicability concerns	High risk	Storage temperature: High risk Conservation: High risk Time: High risk	High risk	—
Kerolus[8]	Risk of bias	Low risk	Storage temperature: Low risk Conservation: Low risk Time: Low risk	?	Low risk
	Applicability concerns	High risk	Storage temperature: Low risk Conservation: High risk Time: High risk	High risk	—
McKnight[9]	Risk of bias	?	Storage temperature: High risk Conservation: ? Time: Low risk	?	?
	Applicability concerns	High risk	Storage temperature: Low risk Conservation: ? Time: High risk	High risk	—
McGill[10]	Risk of bias	?	Storage temperature: ? Conservation: High risk Time: High risk	?	?
	Applicability concerns	High risk	Storage temperature: Low risk Conservation: High risk Time: High risk	High risk	—
Gálvez[11]	Risk of bias	Low risk	Storage temperature: Low risk Conservation: Low risk Time: Low risk	?	Low risk
	Applicability concerns	High risk	Storage temperature: Low risk Conservation: Low risk Time: Low risk	?	—
Tausche[12]	Risk of bias	Low risk	Storage temperature: Low risk Conservation: Low risk Time: Low risk	?	Low risk
	Applicability concerns	High risk	Storage temperature: Low risk Conservation: High risk Time: High risk	Low risk	—
Kienhorst[13]	Risk of bias	Low risk	Storage temperature: Low risk Conservation: Low risk Time: High risk	?	High risk
	Applicability concerns	High risk	Storage temperature: High risk Conservation: High risk Time: High risk	?	—
Present study	Risk of bias	Low risk	Storage temperature: Low risk Conservation: Low risk Time: Low risk	Low risk	—

QUADAS-2: Quality Assessment of Diagnostic Accuracy Studies 2.

MSU crystals form and deposit. Urate probably requires a complementary structure (most likely proteins) to crystallize as MSU[26]. *In vitro* studies have suggested that gammaglobulins or collagen serve as this kind of template[27], with the latter explanation a firm candidate considering the usual deposition of MSU crystals on the cartilage surface, as seen by ultrasound[28] or arthroscopy or in SF fragments[29].

Regarding CPP crystals, we observed a significant decrease in the persistence of crystals in study samples, especially when stored at room temperature and likely when preserved with EDTA. Despite being widely noted in published reviews[30,31], just 1 study supports the effect of EDTA as a solvent for CPP crystals. Bennett, *et al*[32] analyzed the influence of several factors (pH, crystal size, citrate, albumin, and others) on solubility of synthetic CPP crystals under 37°C. Regarding pH, higher solubility was seen at pH 8.0-9.0. Smaller CPP crystals appear to dissolve more quickly. Increasing ionized calcium concentrations decreased CPP solubility, while for ionized inorganic pyrophosphate (iPP), solubility rates followed a J-shaped curve,

being lower at normal SF values (2-25 μM). Higher concentrations likely induce dissolution by Ca++ chelation. iPP hydrolysis by pyrophosphatases also increased CPP solubility. EDTA played a key role here, considering its known effect as a calcium chelator[33]. Our results are in keeping with this observation, indicating that the best storage method for SF samples with CPP crystals is refrigeration and preservation with heparin. The potential applicability of this finding to clinical practice, where CPP crystals cannot be dissolved and the management of CPP crystal arthritis is based only on controlling the inflammatory manifestations[34], needs to be further addressed. In the study by Bennett, *et al*[32], when EDTA was applied to patients with CPP through joint lavages, it triggered severe, acute CPP flares.

The quality of the evidence presented here is strengthened by rigorous efforts to reduce observer bias through masking of the samples (random numbered labeling, control samples with no crystals), including preventing comparisons between observations until study end. Moreover, the observer underwent short training sessions in SF analysis at the beginning of the study and periodic reviews throughout (every 10 samples visualized). No formal reliability assessment was carried out for intra- or interrater agreement, and this might be taken as a limitation, especially because the observer was a medical student. However, polarized light microscopy for crystals is a reliable technique, as reported by rheumatologists[35,36], and with laboratory registrars after brief training[14]. In our study, similar training was followed before starting the study and repeated during it; proper crystal identification was verified at these times. Moreover, the observer properly identified the control samples containing no crystals. These strategies likely ensure the value of the study data, which was later confirmed in the multivariable analyses; they were identical regardless of incorporating the T0 visualizations. Because observations were performed on consecutive days, the observer might expect progressive crystal degradation; however, tubes were masked using nonconsecutive enumeration, and while noting significant differences with either refrigerated or MSU crystals, EDTA-containing tubes indicated minimal effects. The sample size of 30 may be considered small and could have had an influence on the nonsignificant results; however, given the separation of the samples into 4 different tubes, results were based on 120 subsamples and 360 observations. In addition, the use of paired samples reduced the sample size needed to detect differences. Despite no formal evaluation, the observer's impression was that the cells present in the samples progressively lysed and died, mainly at room temperature, which can hamper crystal identification (specifically to assess CPP shape). Here, such identification was performed according to standards, although future studies may aim to replicate our results using techniques with higher sensitivity, e.g., centrifugation[37], or methods that eliminate the observer-related variability, e.g., Raman spectroscopy[38]. This research would be of special interest for CPP crystals because of the observed reduction in its detection.

Although early analysis of SF samples is advisable for establishing diagnosis and initiating proper management, our data indicate that visualization may be delayed up to 1 week after sampling. MSU crystals persisted during the study period regardless of the storage method. For CPP crystals, storage with refrigeration instead of at room temperature favored persistence; using EDTA and not heparin as the preserving agent appeared to hamper visualization, but this was not confirmed in the statistical analysis. Avoiding a crystal-proven diagnosis due to the immediate unavailability of microscopy no longer appears to be justified.

ACKNOWLEDGMENT

The authors thank Dr. Samuel L. Whittle for providing the full text of articles for the review; Megan Harris for English-language editing; and Prof. Eliseo Pascual for revising the final version of the manuscript.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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