EXTENDED REPORT

Analysis for crystals in synovial fluid: training of the analysts results in high consistency

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Background: Identification of monosodium urate (MSU) and calcium pyrophosphate dehydrate (CPPD) crystals in synovial fluid samples is diagnostic of gout and CPPD crystal related arthropathy. Various studies have shown poor consistency in results of crystal analysis.
Objective: To determine whether training of the analysts increases the consistency.
Methods: An expert rheumatologist gave a course on crystal detection and identification. The four trained observers then blindly and independently examined synovial fluid samples previously classified by the expert which had been obtained from patients with both crystal arthropathies and other non-crystal related inflammatory joint conditions.
Results: 194 observations were made on 64 synovial fluid samples: 96 without crystals (49.4%), 55 with CPPD crystal (28.4%), and 43 with MSU crystals (22.2%). For crystal detection (presence or absence of crystals), sensitivity was 95.9% and specificity 86.5%. For identification of MSU crystals, sensitivity was 95.3% and specificity 97.2%. For identification of CPPD crystals, sensitivity was 92.7% and specificity 92.1%. The κ index of agreement with the reference standard between the observers was 0.84 for any crystal detection, 0.93 for MSU crystal sample identification, and 0.79 for CPPD crystal sample identification.
Conclusions: For trained observers, the detection and identification of crystals in synovial fluid is a consistent procedure.

Identification of monosodium urate (MSU) and calcium pyrophosphate crystals in synovial fluid samples obtained from inflamed joints allows the precise diagnosis of gout and calcium pyrophosphate dehydrate (CPPD) crystal related arthropathy.

MSU and CPPD crystals show different characteristics of birefringence, and the polarised light microscope is the standard method for synovial fluid analysis in the search for crystals.[1–3] The shape and appearance of MSU and CPPD crystals under conventional light microscopy are different and contribute to their differentiation.[4 5] Although MSU crystals are strongly birefringent and easily seen under polarised light, CPPD crystals are poorly birefringent, and many are not birefringent at all.[6]

Diverse studies have shown that crystal identification suffers from a lack of consistency between different observers. For example, in one study four samples of different synovial fluid were sent to 25 laboratories for analysis and crystal identification, and a sensitivity of 78% for the detection of MSU crystals and only 12% for CPPD crystals was obtained.[7] In another study,[8] 11 different aliquots of synovial fluid were distributed to three laboratories, and discrepancies were found in seven of the samples. In a third study, 16 synovial fluid samples without crystals and other samples with different concentrations of CPPD crystals (n = 41) and MSU crystals (n = 42) were analysed[9]; although the correct identification of crystals increased with their concentration, the investigators reported a sensitivity of 69% and a specificity of 97% for MSU crystals, and 82% and 78% for CPPD crystals. In another study aliquots of synovial fluid containing MSU crystals and other samples of synovial fluid containing materials such as cholesterol crystals or starch particles were sent to 25 laboratories; although all samples with MSU crystals were identified correctly, there was a 24% false positive result as other materials were mistaken for MSU crystals.[10] Finally, in yet another study four samples of synovial fluid were distributed to several clinical laboratories between 1989 and 1996; if MSU crystals were abundant, the rate of false positive results was 0–38%, but this increased to 67% when the crystals were scanty.[11] All these results show a lack of consensus in routine analyses of synovial fluid.[12–17]

The lack of consistency in synovial fluid analysis could be explained either by the inability of the technique to give good quality results, or by observer misinterpretation, as crystal identification requires subjective interpretation. In the latter case, the training of the observer is likely to be a determinant of the results. With other techniques requiring subjective interpretation, proper training of the observers is very important in obtaining satisfactory results.[18]

Our aim in this study was to determine whether previous training of the observers results in consistent detection and identification of MSU and CPPD crystals in synovial fluid samples.

METHODS

Source of the synovial fluid samples

The study was prospective. We used 64 synovial fluid samples from patients with crystal related and other inflammatory arthropathies, collected at the clinics of the rheumatology section of the Hospital General Universitario de Alicante, Spain, between September 2001 and June 2003.

Participants

The participants were four residents in the department of clinic analysis. For the purposes of the study, the participating residents, who had no previous experience in synovial fluid analysis, received the training course described below.

Abbreviations: CPPD, calcium pyrophosphate dehydrate; MSU, monosodium urate

Training course

The course was given by one of us (EP) with experience in synovial fluid analysis. First, the morphological and birefringence characteristics of MSU and CPPD crystals were reviewed in a session, and then aliquots of synovial fluid samples with and without crystals were examined blind by the residents over a three month period, at the end of which the trainer considered that the trainees were capable of identifying both types of crystals properly.

Course guidelines

For teaching purposes, we considered that the analysis for crystals needed to be approached in two consecutive steps:

- *crystal detection*, to ensure that crystals will not be missed;
- *crystal identification*, to determine the identity of the crystals detected in the previous step.

Detection of MSU crystals is best done using an uncompensated polarised microscope, under which all such crystals show strong birefringence. By contrast, only about 20% of CPPD crystals show birefringence (and if present it is also weaker than shown by MSU crystals). CPPD crystals are best seen with an ordinary microscope, which is the preferred tool.[6] These crystals are often intracellular, so cells should be examined carefully with this in mind. Thus, when looking for crystals in synovial fluid, the samples should be observed under both a polarised and a conventional microscope or CPPD crystals may be missed.

If crystals are detected, they should be identified by means of a compensated polarised microscope. The appearance of MSU and CPPD crystals is very different, and with practice observers generally have little difficulty in differentiating them, even with the ordinary light microscope.[19] The most common confounding elements in crystal analysis are the common artefacts, and the trainees were familiarised with these. Apatite was not considered, cholesterol was shown in the training course but was not seen afterwards, and steroid crystals were not present in any of the samples analysed as part of the study.

Study procedures

All samples were examined and classified by the reference rheumatologist (EP) who knew their origin. The samples were then divided into aliquots and blindly and independently observed by the participating residents. They first determined whether crystals were present or absent. Then they identified the crystal type (MSU or CPPD), and recorded their results independently. The results were not unblinded and analysed until the end of the study. All observations were done in the first two hours after extraction of the fluid. All the observations were made on an Olympus BH microscope to 400×.

Statistical analyses

Statistical analyses were carried out using the SPSS for Windows 11.0 (SPSS Inc, Chicago, Illinois, USA). The κ index was used for the analysis of concordance (statistical package STATA 8.0); the degree of concordance was expressed as a numerical value for κ, which ranges from 0.0, indicating absolute discordance, to 1.0, indicating perfect concordance. (A value over 0.61 indicates that the agreement is good.)

RESULTS

Samples

In all, 64 samples of synovial fluid were analysed. The clinical diagnoses of the patients were gout (12 patients), CPPD related arthropathy (16 patients), rheumatoid arthritis (12 patients), and other inflammatory arthritis, including juvenile idiopathic arthritis, psoriatic arthritis, spondyloarthropathies, and unclassified polyarthritides (24 patients). The mean (SD) time between obtaining the sample and the evaluation was 48 (54) minutes.

The four analysts made a total of 194 observations on synovial fluid, 96 observations on samples without crystals (49.4%), 55 observations on CPPD crystal samples (28.4%), and 43 observations on MSU crystal samples (22.2%).

Participants

Not all the observers examined the 64 samples: observer 1 examined 38 samples (59.4%), observer 2 examined 56 (87.5%), observer 3 examined 44 (68.8%), and observer 4 examined 56 (87.5%). Thus 20 samples (31.3%) were analysed by all four observers; 30 (46.8%) were analysed by three observers; 10 (15.6%) were analysed by two observers; and four (6.3%) were analysed by only one observer.

Crystal analysis

Crystal detection (presence or absence of crystals)

After examining each preparation with both ordinary light and an uncompensated polarised microscope, we obtained a sensitivity of 95.9%, a specificity of 86.5%, and 13 false positive results (6.7%). The false positives were 10 samples without crystals identified as containing CPPD crystals, and three samples without crystals identified as containing MSU crystals. The values for each observer are summarised in table 1.

Crystal identification

There were 98 observations on synovial fluid samples containing crystals. In the 43 observations on MSU crystal samples, we obtained a sensitivity of 95.3% and a specificity

Table 1 Sensitivity and specificity for the crystal detection (to determine the presence or absence of crystals) in 64 synovial fluid samples

	Observer 1	Observer 2	Observer 3	Observer 4	Total
TP	21	28	19	26	94
TN	13	25	21	24	83
FP	4	3	2	4	13
FN	0	0	2	2	4
Sensitivity (%)	100.0	100.0	90.5	92.8	95.9
Specificity (%)	76.7	89.3	91.3	85.7	86.5
PPV (%)	84.0	90.3	90.5	86.6	87.9
NPV (%)	100.0	100.0	91.3	92.3	95.4

FN, false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

Table 2 Index of agreement (κ) between the four observers with reference standard for detection and identification crystal

Observer	Agreement (%)	Expected agreement (%)	κ	Se	CI
1	86.84	33.45	0.80	0.11	0.58 to 1.02
2	96.43	36.10	0.94	0.09	0.76 to 1.13
3	90.91	39.36	0.85	0.11	0.63 to 1.06
4	89.29	36.54	0.83	0.09	0.64 to 1.01

CI, confidential interval, Se, statistical error.

of 97.2%. In the 55 observations on CPPD crystal samples, we obtained a sensitivity of 92.7% and a specificity of 92.1%.

Agreement between observers

The κ index of agreement between the observers with the reference standard was 0.85. In crystal detection, κ was 0.84; in crystal identification κ was 0.93 for MSU crystal samples and 0.79 for CPPD crystal samples (table 2).

DISCUSSION

Our data show that when observers have been trained in crystal detection and identification in synovial fluid samples, their results are consistent. The two step procedure that we use (crystal detection followed by identification of the crystal detected) may be a determinant of these good results. Previous studies have shown that problems with identifying CPPD crystals are relatively common. Such crystals are often non-refringent[6] and their detection relies on morphological identification by ordinary light microscopy. MSU crystals, on the other hand, are easily detected under uncompensated polarised microscopy, where they shine brightly on the dark field.[4] Compensated polarised microscopy allows adequate identification of detected crystals. Our good sensitivity and specificity for crystal detection appears to support the two step scheme for searching for crystals. It should be borne in mind that with rare exceptions the crystals responsible of arthritis are only of these two types.[14] Artefacts are an important confusing element for those inexperienced or untrained in synovial fluid analysis.

In interpreting our results, it is relevant that the observers had no previous experience with synovial fluid analysis, and that they carried out the observations that form the basis of this study only after a period of formal training. We feel that with additional experience the results would have been better. In fact, most of the misclassification occurred in the initial observations. Identification of the crystals by an expert was taken as the gold standard, and our results would have been strengthened if his consistency had been determined by having him carry out a blind assessment of the samples after their initial classification. Also, the samples studied originated in patients from our clinics and none of them contained cholesterol or corticosteroid crystals. The participants had been familiarised with the usual artefacts and they did not present any problem.

Our sensitivity and specificity for crystal identification was higher than that found in previous studies. For MSU containing synovial fluid samples, we obtained a sensitivity of 95.3% and a specificity for proper identification of 97.2%, in comparison with a sensitivity of 69% and a specificity of 97% found previously.[9] For CPPD crystals we found a sensitivity of 92.7% and specificity for identification of 92.1%; in a previous study, a sensitivity of 12% was found.[7] Another study produced better results, with a sensitivity of 82% and a specificity of 78%.[9] The experience in crystal analysis and the level of training of the observers participating in those studies was not mentioned in the reports. Although we had some false positive results (13 (6.7%) in all), these were fewer than previously reported.[10 11]

Final identification of the detected crystals was recorded only after observation with both ordinary and compensated microscopes, and we did not record a tentative identification at the detection step. Nevertheless, it is our feeling that on most occasions the type of the crystal was already clear after observation with an ordinary light microscope, as reported previously.[19] CPPD crystals are easily identified by their rhomboidal or parallelepipedic shape; only acicular CPPD crystals are likely to be mistaken for the needle shaped MSU crystals, and it is here that the compensated polarised microscope has a definitive role.[13] None of the samples included in this report contained both MSU and CPPD crystals. According to our results, if a polarised light microscope is not available, a trained observer should be able to achieve satisfactory CPPD and MSU crystal detection using an ordinary light microscope.

Our results showed a high level of concordance between the observers after their training and the expert who had classified the synovial fluid samples for the study. The global κ value was 0.85 (because the number of ratings per subject varied, we could not calculate the individual test statistics). Concordance was highest for MSU crystals ($\kappa = 0.93$) and less for the samples with CPPD crystals ($\kappa = 0.79$), emphasising again that the main problem lies in identifying the latter type of crystal.

A quality control programme in crystal identification needs to be initiated, with special attention to the sensitivity and specificity of the method. Ideally such a programme would involve both the training of the analysts, and assessment of the examination procedure. Quality control programmes are now used to monitor most laboratory tests, but interest shown in synovial fluid analysis by clinical laboratories has been low, and crystal analysis is not established as a sound routine in most departments. Rheumatologists themselves appear to carry out most of the analyses of the fluids they draw; so formal training would seem to be essential to assure consistency of their results.

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