Most calcium pyrophosphate crystals appear as non-birefringent

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Abstract

Objective—To determine the proportion of calcium pyrophosphate dihydrate (CPPD) crystals that appear as non-birefringent when observed under the polarised light microscope.

Methods—Two observers examined independently 10 synovial fluid samples obtained during an episode of arthritis attributable to CPPD crystals. Ten synovial fluid samples from patients with acute gout were used as a reference. The examination was performed after placing a fluid sample in a Niebauer haemocytometric chamber; a crystal count was done first under ordinary light, then in the area corresponding to a 0.1 ml, under polarised light.

Results—The percentages of birefringence appreciated for CPPD were 18% (confidence intervals (CI) 12, 24) for observer 1, and 17% (CI 10, 24) for observer 2 (difference NS). The percentages of birefringence for monosodium urate were 127% (CI 103, 151) for observer 1, and 107% (CI 100, 114) for observer 2 (difference NS). Percentages above 100% indicate that crystals missed under ordinary light became apparent under polarised light.

Conclusion—Only about one fifth of all CPPD crystals identified by bright field microscopy show birefringence when the same synovial fluid sample is observed under polarised light. If a search for CPPD crystals is conducted under polarised light, the majority of the crystals will be missed. Ordinary light allows a better rate of CPPD crystal detection but observation under polarised light of crystals showing birefringence is required for definitive CPPD crystal identification.

The gold standard for the precise diagnosis of joint inflammation in gout and calcium pyrophosphate dihydrate (CPPD) crystal arthropathy requires finding either monosodium urate (MSU) or CPPD crystals in synovial fluid (SF) samples obtained from inflamed joints. The standard procedure for the identification of these crystals requires the use of a polarised microscope fitted with a first order red compensator. The different shape of the crystals (MSU crystals being always acicular, and acicular, rhomboid or parallelepipedic in the case of CPPD) and the type of elongation (strong negative for MSU and weakly positive for CPPD) allows a proper identification. MSU crystals are strongly birefringent when seen with the microscope fitted with crossed polarised filters, and acquires a negative elongation when the first order red compensator is used. On the other hand, CPPD crystals are described as being only weakly birefringent and it has been noted that some CPPD crystals may lack birefringence.

The process of crystal search in SF requires: firstly, to determine whether the fluid contains crystals, and then proper crystal identification. As the compensated polarised microscope is the standard for crystal identification, it is probably also widely used to determine whether a SF sample contains crystals or is free of them. As CPPD crystals are weakly birefringent, and ON occasions may be non-birefringent, these crystals may pass unnoticed if their search is conducted in this manner. In this context our previous experience has been that a number of CPPD crystals appear as non-birefringent and pass unnoticed in the darker polarised microscope field, while are seen well with ordinary light. Our aim in this paper is to quantify the percentage of CPPD crystals that appear as non-birefringent under polarised light in SF samples obtained from patients with CPPD related joint inflammation, and consequently could easily pass unnoticed if searched for in this manner. We have used SF samples from patients with acute gouty arthritis for comparison.

Methods

SYNOVIAL FLUIDS SAMPLES

We obtained 10 SF samples from 10 different knees of different patients previously diagnosed with CPPD related arthropathy after identification of CPPD crystals in their SF. All had had at least one previous episode of joint inflammation, and all had typical radiological condrocalcinosis in their knees. As controls, we used 10 SF samples from 10 different knees with acute gouty arthritis in patients previously diagnosed with gout. In all but one the diagnosis had been previously established by
identifying MSU crystals in their SF, in the remaining one the diagnosis was established after examining an aliquot of the sample that was then submitted to study. None of the SF samples had coincident CPPD and UMS crystals and none of the investigated joints had been injected previously with corticosteroids. The samples were obtained and provided by other members of the staff, after ascertaining their suitability for the study, and the observers were not informed of the type of crystal.

PROCEDURE
An aliquot of each SF sample was independently examined within the first two hours of its collection by two senior residents in rheumatology (observers 1 and 2) with ample experience in SF analysis and crystal identification. A sample of each SF was placed undiluted in a Niederhauser haemocytometer chamber where the crystals—intracellular and extracellular—present in a volume of fluid corresponding to 0.1 ml were counted; if the samples were felt to be too cellular for a crystal count, the SF was diluted 1/10 in 0.9 saline, as described previously. The observations were made with an Olympus BH microscope fitted with a halogen light source, and at 400× magnification.

A crystal count was done first under ordinary light—where all crystals identified were counted—and then it was repeated in the same fields under uncompensated polarised light, and all crystals identifiable because of their birefringence were noted. A final observation after placing the first order red compensator was made for definitive crystal identification.

STATISTICS
The percentage of birefringent crystals was calculated for each SF sample (number of crystals seen under ordinary light/number of crystals seen under polarised light × 100). The mean percentages of birefringent crystals seen by each observer were calculated. For statistical analysis we used the Mann-Whitney test. Results have been expressed as means and confidence intervals.

Results
Under ordinary light, observers 1 and 2 counted a total of 949 and 815 CPPD crystals respectively and 232 and 219 MSU crystals.

The percentages of birefringence for CPPD were 18% (CI 12, 24) for observer 1, and 17% (CI 10, 24) for observer 2 (difference NS). In none of the samples was this percentage above 40% by either of the observers.

The percentages of birefringence for MSU were 127% (CI 103, 151) for observer 1 and 107% (CI 100, 114) for observer 2 (difference NS). The percentages above 100% are because of the fact that some MSU crystals passed unnoticed under ordinary light, and became apparent when crossing the polarised filters. Very occasional MSU looking crystals did not show birefringence. In none of the samples with MSU crystals was the percentage of birefringence below 80%.

The difference of percentages of birefringence between CPPD crystals and MSU was statistically significant (p<0.001) considering together the results of both observers.

Extracellular CPPD crystals (seven samples) and intracellular ones (nine samples) showed birefringence percentages of 7.8% (CI 4, 11) and 16% (CI 10, 22) (difference NS). We also failed to find a relation between the number of crystals per sample and the percentage of birefringence (r=0.205, p>0.05).

Discussion
In this study we have found that only about one fifth of all CPPD crystals identified by bright field microscopy show birefringence when the same SF sample is observed under polarised light—uncompensated—at 400× magnification. In this setting, a crystal showing birefringence is distinguished—and thus detected—shining above the darker microscope field when observed with a microscope fitted with crossed polarised filters. Thus, if birefringence is the characteristic that is considered to allow detection of CPPD crystals in a SF sample, a majority of these crystals will pass unnoticed if the search is conducted under polarised light. Also, CPPD crystals detected by their shape if the search is conducted under ordinary light may be dismissed as artefacts because of their lack of birefringence under polarised light microscopy. We did not attempt to quantify the percentage of crystals lacking birefringence under uncompensated polarised light, but we found that those CPPD crystals not showing birefringence under polarised light did not gain it when a first order red compensator was added to the system (simply, with compensated polarised light, the crystals, as any other structure, were better distinguished by their shape because the background of the microscope field was brighter). It should be remembered that the use of a first order red compensator helps to distinguish between the positive and negative type of the elongation shown by birefringent materials. In the setting of SF analysis the use of a compensator essentially facilitates the distinction between MSU crystals that show strong negative elongation and CPPD crystals that show a weaker positive elongation, and also facilitates the distinction of other crystals or artefacts showing different birefringent characteristics.

In the SF samples containing MSU crystals, which were used as controls, the number of crystals seen when the polarising filters were crossed outnumbered those initially detected under ordinary light; this indicates that polarised light microscopy is a more appropriate mean for the detection of MSU crystals, and adds weight to our observations on CPPD crystals.

The observations were conducted in parallel by two senior rheumatology residents with experience in crystal identification in SF, and both obtained comparable results with CPPD as well as with MSU crystals. The observers were blinded to the type of crystal present in the SF; in all cases the crystals were easily and properly identified, and no SF contained both types of crystals. The halogen light source of the microscope provided ample illumination. Probably using different microscopes, polarised
filters, light sources or just an observation made by different persons may result in different percentages of appreciated birefringence for the CPPD crystals. But it also seems probable—although this has to be checked—that even with different systems/observers, a large percentage of CPPD crystals may still lack enough birefringence to pass unnoticed. We did some unquantified observations of samples containing CPPD crystals with two other different microscopes, and also found an ample percentage of apparently non-birefringent crystals.

The weaker birefringence shown by CPPD crystals was already noted by McCarty in the original paper in which the crystals were described, whereas to increase the sensitivity for CPPD crystal detection, a phase contrast microscope as well as compensated polarised filters were used; this system has remained the standard tool for searching for crystals by this author. This weak and occasionally absent birefringence of CPPD has been also noted by others. As shown in the results, in the SF samples in which this was quantified, absence of birefringence was equally frequent in intra-cellular and extracellular crystals. The percentage of non-birefringent crystals was also independent of the number of crystals per sample. We did not investigate whether CPPD crystals of different sizes showed different percentages of apparent lack of birefringent, but both large and small crystals were frequently non-birefringent; the possibility that smaller crystals may appear more frequently as non-birefringent remains open.

It can be argued that some of the structures considered to be CPPD crystals under regular light in this paper may have been artefacts; we only considered as crystals structures with clearly defined edges and regular geometric rod, rhomboid or parallelepiped shape. Besides, those showing birefringence and those not showing it were indistinguishable under ordinary light. The possibility of identifying CPPD by means of an ordinary light microscope has been documented. Although we did our observations at 400 magnification, observation at 1000 magnification probably helps in the detection of smaller CPPD crystals by allowing a better distinction of their edges, but birefringence at this magnification may be less apparent.

Of particular interest to clinicians, different studies have found poor reproducibility in crystal detection and identification between different laboratories, both in relation to CPPD crystals as MSU. In one such study CPPD crystals were correctly identified only on six occasions out of 50 observations of SF that contained them; the authors commented that the crystals could have been missed because of the quality of the microscopes used by the clinical laboratories. In other studies CPPD crystals were also frequently missed or misinterpreted. In a third report in which the reliability for crystal detection was explored, observations were made by means of a polarised microscope fitted with a first order red compensator: the authors found less difficulty in detecting/identifying MSU crystals than CPPD crystals. All these studies have found a worrying poor reproducibility in identifying crystals, and at least for CPPD crystals the frequent lack of birefringence reported here may at least partially account for it. The need for quality control in crystal analysis has recently been emphasised.

MSU and CPPD crystal identification in SF are the gold standards for the diagnosis of gout and CPPD related arthropathy. Establishing the diagnosis of these diseases often implies important therapeutic decisions, and it is essential to assure the widespread use of proper techniques for crystal detection and subsequent identification. Only crystals that have been detected can be identified; although the polarised microscope is an important tool in differentiating MSU from CPPD crystals and other rarer crystals or some artefacts, according to our results, it is a poor tool for CPPD crystal detection—as a majority of non-birefringent crystals may pass unnoticed. When CPPD crystals are suspected, observation of the SF sample with ordinary light (at 400 or at 1000×), or phase contrast microscopy, may help to detect the crystals in the majority of the cases in which they are present: if crystals are detected, they should be always evaluated under polarised light for definitive identification, although it should be kept in mind that only some of the crystals will show birefringence. Polarised microscopy, compensated or uncompensated seems superior to ordinary light microscopy for the detection of MSU crystals.

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