Identification of crystals in synovial fluid

Synovial fluids can contain a number of crystals and other particulate matter. Some of these, particularly monosodium urate monohydrate (MSUM) and calcium pyrophosphate dihydrate (CPPD) crystals, are pathogenic; others, including cholesterol and other lipid particles and the basic calcium phosphates (BCPs), including apatites, are of doubtful significance. Table 1 lists the main forms of particulate matter that have been identified in synovial fluids.

In the case of MSUM and CPPD crystals it is clear that the identification of these crystals in a synovial fluid that also has a high polymorphonuclear cell count (indicative of acute inflammation) is the only certain way to diagnose an attack of gout or pseudogout respectively.1 Furthermore, this is one of the few tests that has been shown to change clinical practice in rheumatology,2 and it is apparent that the consequences of getting the diagnosis wrong can be severe.3 Therefore, accurate identification of MSUM and CPPD is important.

Table 1: Intrinsic and extrinsic crystals and particles detected in synovial fluid

<table>
<thead>
<tr>
<th>Pathogenic or Non-pathogenic</th>
<th>Extrinsic crystals or particles (introduced from outside)</th>
<th>Intrinsic crystals or particles (formed within the joint)</th>
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</thead>
<tbody>
<tr>
<td>Corticosteroid can cause short lived inflammatory episode</td>
<td>From air - dust, fungal spores, (lens) tissue fibres, etc; many are birefringent</td>
<td>Non-birefringent 1–20 µm “broken glass” cartilage in all SF</td>
</tr>
<tr>
<td>Storage artefacts - Brushte, +vely birefringent rods 10 µm+ in “starburst”</td>
<td>CPPD + birefringent rods/homoids, 1–20 µm. Can form “beachballs”</td>
<td>Non-birefringent 1–20 µm “broken glass” cartilage in all SF</td>
</tr>
<tr>
<td>“Drying-out” SF gives spectacular birefringent particles resembling MSUM, CPPD, etc</td>
<td>BCP - submicroscopic, very weakly birefringent. Aggregates 2–80 µm visible if stained.</td>
<td>Larger (100 µm+) birefringent “silk-sheen” cartilage in damaged/arthritic joints.</td>
</tr>
<tr>
<td>Ovoid starch granules from gloves, 10–100 µm + birefringent weak “Maltese cross”</td>
<td>CPPD + BCP (often)</td>
<td>Meniscal fragments = silk-sheen + fibrillar appearance.</td>
</tr>
<tr>
<td>Corticosteroids (all strongly birefringent crystals 1–40 µm)</td>
<td>CPPD + BCP (often)</td>
<td>Fragments many contain CPPD/MSUM</td>
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<tr>
<td>Lederspan (1–10 µm) resembles CPPD</td>
<td>MSUM + CPPD</td>
<td>Fragments of fibrillar cruciate ligament - joint injury</td>
</tr>
<tr>
<td></td>
<td>MSUM + CPPD</td>
<td>Fragments of synovium - villi + birefringent fibrous core</td>
</tr>
<tr>
<td></td>
<td>MSUM + cholesterol, etc</td>
<td>Rice bodies - aggregates of synovial tissue in RA SF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipid crystals ~ 1–30 µm ovoids. Birefringent. Bright “Maltese cross”</td>
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<tr>
<td></td>
<td></td>
<td>Cholesterol 5–40 µm + birefringent notched plates. Lipid globules - non-birefringent (resemble air bubbles)</td>
</tr>
</tbody>
</table>

Figure 1: Monosodium urate monohydrate crystals from a gouty tophus viewed under polarised light. Note the strong birefringence, needle shaped morphology and “flaring” that help distinguish them from other crystals.
and is relatively inexpensive; in addition, as MSUM and CPPD crystals can be distinguished through their different polarisation properties, it should, in theory, be reasonably reliable for these two crystal types (figs 1 and 2). But polarised light microscopy is not a crystallographic technique (and therefore cannot permit certain characterisation), and it is virtually useless for the identification of basic calcium phosphates (BCP). However, we have yet to be convinced that the identification of apatite or other BCP particles in the synovial fluid ever should or does affect patient management, which renders it irrelevant to clinical practice.

We therefore recommend that clinical rheumatologists should only use polarised light microscopy to identify MSUM and CPPD crystals, and to differentiate them from “look-alikes”, while ignoring the issue of BCP crystals and all other techniques for crystal identification.

Is routine polarised light microscopy reliable?

No.

In the past it has often been the case that writers of reviews and textbooks (other than our own) have indicated that the identification of MSUM and CPPD by polarised light microscopy is simple and easy, when in fact it is not. The veneer of simplicity may arise from the fact that it tends to be those with skill and experience who write the reviews, and because the visualisation of a sample packed full of urate needles is both simple, seductive and immensely satisfying.

However, several quality control exercises have indicated that inter-laboratory reliability is very poor. When synovial fluid samples containing a variety of different crystals have been passed around different laboratories every type of error has commonly occurred—false negatives, false positives and misclassification—clearly the technique lacks both sensitivity and specificity. We have a problem.

Why is polarised light microscopy unreliable?

There are a number of reasons for the problem.

The lack of sensitivity is largely attributable to the fact that crystals may be either too small (individually) or too sparse (low concentration) to be identified. There is evidence in support of each of these contentions. Gordon et al. added varying concentrations of crystals to synovial fluids and found that microscopists had an identification threshold of around 10–100 μg/ml, whereas experiments with extraction have shown that in vivo concentrations may be as low as 2–3 μg/ml. In addition, we and others have found that CPPD crystals range in length from around 0.4 to 20 μm, and that the majority are sometimes below the 1 μm threshold that would allow identification by light microscopy. This explains false negatives. False positives probably arise from the misclassification of contaminants as MSUM or CPPD crystals, and is therefore a matter of skill and experience.

The lack of specificity, leading to misclassification of particles, can also be explained by lack of experience and skill. More problems arise with CPPD than with MSUM. CPPD crystals are only weakly birefringent, and are probably better identified by non-polarised light and characterised by morphology than by their polarisation properties.

What can and should we do about the problem?

We must get our house in order.

Recent headlines about other laboratory tests, such as cervical smears, indicate that the widespread use of a test that changes patient management, but is being carried out with a lack of specificity and sensitivity will no longer be tolerated. Nor should it. We must get our house in order.

The sensitivity problem cannot be solved without the routine use of very complex techniques such as crystal extraction from synovial fluids and analytical electron microscopy—and this is impractical. However, we can and should always remember that the presence of crystals in synovial fluids is not an “on-off” phenomenon and that there is a threshold for identification. In practice, it is unlikely that an episode of gout or pseudogout will generally be driven by crystals that are either too small or too low in concentration to be identified during the episodes, but this will often be the case between acute episodes of synovitis. As rheumatologists we must recognise that a negative report from the examination of a synovial fluid by polarised light microscopy, however experienced the operator, does not exclude the presence of small numbers of MSUM or CPPD crystals, or of many very small crystals of these salts.

The specificity problem is one that we can do something about. This revolves around experience, technique, equipment and skills. The service could, in our view, be greatly improved by making sure that laboratories that take on this work have a good microscope, a specially trained and experienced person to carry out the microscopy, and that they carry out good practice in this field. This is something that EULAR has helped to kick start and that we and others are hoping to continue.

The EULAR initiative

During and after the Amsterdam EULAR Meeting (1993), disquiet was voiced about the poor quality of routine polarised light microscopy for the identification of MSUM and CPPD. As a result, a small grant was awarded to Elisio Pascual and ourselves to kick start an initiative to develop training, guidelines and quality control in this field. The products to date include a published booklet, which will soon be available on the EULAR web site, the completion of a number of training days (including one at the Vienna EULAR meeting in 1997), and similar courses are now being put on by Ralph Schumacher in the USA among others. We are now progressing towards establishing a quality control laboratory in Bristol. We intend to use this to make samples available to collaborating laboratories for quality control, as well as developing and implementing good practice guidelines, and carrying out training, as occurs in many other laboratory practices. Contact us if you would like to be a part of this ongoing work.

Conclusions

The only crystals worth looking for in synovial fluids are MSUM and CPPD, as these are the only ones whose identification can and should change clinical practice.
• Polarised light microscopy remains the only practical way of identifying these particles in the clinical setting.
• Polarised light microscopy is a test with major problems in both its sensitivity and specificity for the identification of these crystals.
• The sensitivity problem is in part attributable to the problems caused by low concentrations of crystals or the presence of crystals of very small size, a negative report could always be a false negative therefore, and crystal presence cannot be regarded as an “on-off” phenomenon.
• The specificity problem can be improved by the use of good equipment, good laboratory practice, specific training and quality control exercises—initiatives to develop these improvements need supporting.

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Unusual and memorable
Series editor: Gary D Wright

A 39 year old man was referred with increasing swelling of the right hallux, and a similar, smaller swelling on the left side. This was progressive over the preceding 12 months, and was painful on weight bearing. There was marked, diffuse soft tissue swelling, tenderness, slight restriction in joint range and associated nail changes (fig 1). Plain radiograph (fig 2) suggested bone proliferation, and computed tomography was requested (fig 3). These show florid bony excrescences arising from the terminal phalanges of both halluces, thickened cortices, normal medullary cavities, and extensive soft tissue swelling.

Periostitis and bone proliferation may be associated with soft tissue swelling and nail changes on the same digit, and can be an early feature of psoriatic arthritis (and other spondyloarthropathies) before significant joint involvement occurs. Periosteal bone may entirely cloak the cortical surface (particularly of the terminal phalanges of the toes), and when associated with trabecular thickening can cause the entire phalanx to appear radiodense, the so called “ivory phalanx”.


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