EDUCATIONAL COMMENTARY – SYNOVIAL FLUID CRYSTALS: MONOSODIUM URATE, CALCIUM PYROPHOSPHATE, AND CHOLESTEROL

Learning Objectives
Upon completion of this exercise, participants will be able to:

• describe the correct procedure for examining synovial fluid for the presence of crystals.
• differentiate among monosodium urate, calcium pyrophosphate, and cholesterol crystals with regard to shape and appearance using polarized microscopy.
• discuss the clinical significance of monosodium urate, calcium pyrophosphate, and cholesterol crystals in synovial fluid.

Synovial fluid analysis for the presence of crystals is performed to evaluate disease associated with the movable (synovial) joints. The fluid is contained in a cavity located between the cartilage-lined bones of the movable joints. It is a viscous liquid produced as a plasma ultrafiltrate containing hyaluronic acid contributed by the cells (synovocytes) of the synovial cavity membrane.

Disruption of the normal ultrafiltration and the production of hyaluronic acid, as well as the presence of foreign substances such as crystals and bacteria, results in pain and swelling in the affected joint. To determine the cause of this condition, synovial fluid is frequently collected by arthrocentesis and sent to the laboratory for crystal evaluation.

Examination for Synovial Fluid Crystals
Fluid for crystal examination is routinely collected in a clean, sodium heparin tube. It is important to avoid contamination of the specimen with powdered or oxalate anticoagulants. Examination of the fluid should not be delayed because the formation and solubility of crystals are affected by temperature and pH. In addition, certain crystals are phagocytized by leukocytes. The presence of phagocytized crystals is a helpful identifying characteristic. If the examination is postponed, the leukocytes may lyse before these characteristics can be observed.

The microscopic examination of synovial fluid is performed as a wet preparation. A drop of synovial fluid is placed on a precleaned glass slide and covered with a clean coverslip. It is important to ensure that the slide and coverslip are clean and not scratched because many artifacts (such as talc and starches from gloves, scratches and nicks on the glass slide, and airborne contaminants) can resemble crystals, particularly under polarized light.

Ideally, the initial examination of the wet preparation is performed using direct and compensated polarized light. It may be possible to see the crystals under brightfield microscopy, but there is a definite possibility of missing or misidentifying them. Phagocytized crystals may be observed on a Wright- or Gram-stained slide, but these techniques should not replace the polarized microscopy examination.
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Direct and Compensated Polarized Microscopy
The ability of these synovial fluid crystals to be viewed under polarized light increases the sensitivity of their detection. Crystals capable of polarizing light are referred to as being birefringent. This means that they have the ability to split a single beam of light into 2 beams rotating 90° from each other. A crystal that rotates the beam clockwise is said to have positive birefringence; a crystal that rotates the beam counterclockwise has negative birefringence. Both types of birefringence produce a white object against a black background. This terminology can be confusing when examining monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals. Monosodium urate crystals are described as having negative birefringence; however, under direct polarized light they appear much brighter than the CPPD crystals which are referred to as having positive birefringence. Fortunately, the degree of brightness is not a confirmatory feature. After a MSU or CPPD crystal is observed, compensated polarized microscopy can confirm the crystal identification.

Examination under compensated polarized microscopy is recommended to confirm the identity of MSU and CPPD crystals. By inserting a first-order red compensator above the polarizing filter, the polarized light beam is split into slow- and fast-moving rays (vibrations) and produces a red background. The compensator is marked with an arrow indicating the direction of the slow vibration. Based on the molecular structure of the crystal and its positive or negative birefringence when it is aligned parallel to the direction of the slow vibration, it will produce either a yellow or blue color against the red background. The color will be reversed if the crystal is aligned perpendicular to the direction of the slow vibration. These color changes are used to confirm the identification of MSU and CPPD crystals.

Monosodium Urate Crystals
Inflammations caused by the presence of MSU crystals are diagnosed as gout. Gout is associated with increased levels of uric acid in the body. Conditions that cause these increased levels include increased destruction of nucleic acids as occurs during chemotherapy for myeloproliferative diseases, and decreased renal excretion of uric acid. Serum uric acid is often elevated but is not as diagnostic as the observation of MSU crystals in the synovial fluid.

Monosodium urate crystals appear as thin, needle-shaped structures with pointed ends. They can, however, appear as short rods and fragments. They do not appear rhomboid or square. They can be seen both intracellularly and extracellularly. Because of their length and pointed ends, they are frequently seen protruding from the phagocytizing neutrophils. Monosodium urate crystals exhibit negative birefringence. Under direct polarized light, they appear very bright against the black background. Under compensated polarized light they appear yellow when aligned parallel to the slow vibration and blue when aligned perpendicular to the slow vibration. In acute attacks of gout, crystals aligned in both directions
are often seen in the same microscopic field. Identification of MSU crystals with compensated polarized light can be considered a definitive diagnosis for gout.

**Calcium Pyrophosphate Dihydrate Crystals**

Calcium pyrophosphate dihydrate crystals cause inflammations similar to those of gout. The condition is called pseudogout because of the difference in crystals and the source of the crystals. The CPPD crystals are associated with degenerative bone and cartilage disorders including articular cartilage calcification (chondrocalcinosis), osteoarthritis, and endocrine disorders that cause elevated calcium levels.

CPPD crystals characteristically exhibit rhomboid or square shapes, although they may appear as short rods. They are easily phagocytized and are frequently seen within the phagolysosomes (vacuoles) of the neutrophils. MSU crystals are not seen within the vacuoles because their sharp ends puncture the phagolysosome membranes.

Calcium pyrophosphate dihydrate crystals are positively birefringent. Under direct polarized light they are not as bright as MSU crystals. When aligned parallel to the slow vibration under compensated polarized light, CPPD crystals are blue. Perpendicular alignment with the slow vibration produces a yellow color. As with MSU crystals, their identity should be confirmed using compensated polarized microscopy.

**Cholesterol Crystals**

Cholesterol crystals are another birefringent crystal that may produce inflammation in a single joint similar to gout and pseudogout. However, they are primarily associated with chronic inflammatory disorders affecting more than one joint (polyarthritis). Disorders associated with polyarthritis include autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, and infections such as Lyme disease.

Similar to the cholesterol crystals seen in urine, synovial fluid cholesterol crystals appear as large, flat plates with notched corners and are highly birefringent under direct polarized light. Cholesterol crystals are too large to be phagocytized. Cholesterol crystals may be accompanied by lipid crystal spheres that exhibit a Maltese cross formation under direct polarized light. These spheres may resemble starch granules, although starch is not usually as sphere shaped. Lipid crystals by themselves also have been associated with acute monoarticular attacks resembling gout and pseudogout.

**Conclusion**

The Table summarizes the three crystals discussed. Detection and identification of crystals in synovial fluid is important for patient diagnosis and subsequent treatment.
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TABLE. Identifying Crystals in Synovial Fluid.

<table>
<thead>
<tr>
<th>Crystal</th>
<th>Description</th>
<th>Polarization</th>
<th>Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSU</td>
<td>Needle-shaped</td>
<td>Highly birefringent</td>
<td>Parallel = yellow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Perpendicular = blue</td>
</tr>
<tr>
<td>CPPD</td>
<td>Rhombic/square</td>
<td>Moderate birefringence</td>
<td>Parallel = blue</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Perpendicular = yellow</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Large, flat plates with notched corners</td>
<td>Highly birefringent</td>
<td>Weakly birefringent</td>
</tr>
</tbody>
</table>

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