EDUCATIONAL COMMENTARY – LABORATORY ANALYSIS OF SYNOVIAL FLUID

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Learning Objectives

On completion of this exercise, the participant should be able to

- describe the function and appearance of normal synovial fluid;
- list two diseases afflicting the joints;
- identify four common cell types in synovial fluid;
- identify three common crystals in synovial fluid; and
- associate three types of crystals with their disease states.

Introduction to Synovial Fluid

Synovial fluid, or “joint fluid,” is normally present in the cavities of movable joints to reduce friction between the bones during movement. Trauma, degeneration, or infection can cause the joint to painfully swell with increased fluid and sometimes cells; crystal formation can also occur. Laboratory analysis can aid the clinician with classification and treatment of pain and stiffness in a joint, collectively termed arthritis.

Joint fluid is produced by specialized cells, called synovial lining cells or synoviocytes, in the membrane lining the joint capsule.¹ The fluid lubricates the joints, provides nutrients to the cartilage, and reduces shock compression from walking and running. It is formed as an ultrafiltrate of plasma across the synovial membrane.² Synovial lining cells secrete into the fluid a mucopolysaccharide containing hyaluronic acid and protein. The hyaluronic acid is what makes the fluid thick and viscous for lubrication. Decreased viscosity is one cause of arthritis in an affected joint.²

Types of Joint Disease

Investigation of joint disease should include routine examination in the laboratory for macroscopic appearance, Gram stain and culture, cell count and differential, and a crystal examination using polarized light microscopy. Occasionally, chemical or immunological analysis may also be desired.¹ The findings will aid in classification of the joint disorder into one of four basic categories: non-inflammatory,
inflammatory (immunologic or crystal-induced), hemorrhagic, or septic. It should be noted that there is some overlap among the laboratory results for the classification groups, specifically the total leukocyte count and differential, so the patient’s clinical history is important.²

Non-inflammatory disorders include age-related degeneration or osteoarthritis. Inflammatory disease can be due to an immunologic disorder such as rheumatoid arthritis, lupus erythematosus, scleroderma, ankylosing spondylitis, rheumatic fever, or Lyme disease arthritis. Inflammation can also occur from crystal-induced gout or pseudogout. Hemorrhagic disorders can be caused by injury, tumors, or coagulation disorders such as hemophilia.¹² Sepsis can be caused by trauma or systemic involvement. The most common organisms found in synovial fluid are *Staphylococcus, Streptococcus, Haemophilus,* and *Neisseria,* as seen in Figure 1. Fungal or viral infections are also possible but not expected.

![Staphylococcus, Streptococcus, Neisseria](Image)

**Figure 1.** Images by James Archer. Courtesy of CDC.

**Macroscopic Examination**

Normal synovial fluid should be clear, yellow, viscous, and less than 3.5 mL aspirated. It should not spontaneously clot. Fluid from a diseased joint may contain fibrinogen and coagulation factors and can clot on its own.² Turbidity usually indicates leukocytosis and/or crystals and increases with the degree of inflammation or infection present.¹ Blood and free fat droplets together are seen in crush injuries or fractures. Blood alone could be from injury or a traumatic tap; a traumatic tap will show streaks of blood in the fluid rather than a homogenous red color. Infected joint fluid will appear turbid or purulent, containing many white blood cells and organisms.¹ Sometimes only a dense mass of crystallized material is aspirated, especially from joints in the fingers, toes, ankles, or wrists. **Figure 2** shows several synovial fluid samples.
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Left to right:
- Green, purulent
- Yellow with crystals
- Dark yellow, small clot
- Orange and cloudy x2
- Bloody, red
- Dark red with clots

Figure 2. Reproduced with permission from PeaceHealth Laboratories.

Viscosity, the polymerization of hyaluronic acid, is also analyzed at this time. Normal synovial fluid should be moderately viscous, to aid in lubrication and cushioning of the joints. Normal fluid should form a string at least 1 ½ inch long when dripped from a pipette or syringe. Fluid of decreased viscosity will fall as free droplets instead of stringing, and is indicative of an arthritic joint. It is possible to measure the amount of hyaluronic acid polymerization by performing the Ropes, or mucin clot, test, but this is very rarely requested as it provides almost no clinically significant information. To perform the mucin clot test, dilute acetic acid is added and the synovial fluid is observed for dissolution of the clot that forms. Normal synovial fluid retains a solid clot. However, it is usually adequate to ascertain whether the viscosity is normal or decreased using the string method, as all forms of arthritis decrease synovial fluid viscosity.

Hematology Cell Counts

Before performing cell counts on synovial fluid, whether by manual hemocytometer or by automated instruments, it is necessary to neutralize the stickiness of the hyaluronic acid by adding the enzyme hyaluronidase. Failure to add hyaluronidase will inhibit correct pipetting for manual counts and will clog an instrument’s apertures. The hyaluronidase should act within 5 minutes and, ideally, the count should be performed within an hour to minimize cell degradation resulting from age. Hyaluronidase itself does not cause cell lysis. Automated cell counters require a fluid to be free of crystals and large debris or clots to prevent clogging and inaccurate counts.

The total leukocyte count is the most significant part of a blood cell count. Red blood cells are typically also included on the report, but the red blood cell count is less useful to the clinician. The presence of a significant amount of red blood cells can be observed macroscopically; a traumatic tap can be differentiated from a hemorrhagic state by determining whether the blood is evenly distributed throughout the fluid (hemorrhage) or appears streaky (aspiration contaminant).
Normal joint fluid should have less than 200 leukocytes/µL, but leukocyte concentration may reach as high as 100,000/µL in massive infections or crystal-induced inflammation. If necessary because of large clots or the presence of crystals, white blood cells and red blood cells can be plated manually on a Neubauer counting chamber (Figure 3). However, recent studies on modern automated cell counters have shown that the automated cell counts are actually more accurate than manual counts, due to improved technology and precise sampling.\(^2\)

**Cell Morphology**

Slide preparation is often the trickiest part of performing a differential count on synovial fluid. In the same manner as the complete blood cell count, the synovial fluid should be treated with hyaluronidase before preparing a thin or cytocentrifuged slide for the differential. If hyaluronidase is omitted, the cells will be too condensed and dark to make an accurate identification on a Wright stain. Some synovial fluid specimens may also have an increased total protein concentration due to inflammation, which can cause the leukocytes to appear too dark and small for identification. This problem can be solved by making a dilution with normal saline, then cytocentrifuging the dilution to ensure enough white blood cells for a 100-cell differential. In some cases, it may also be advisable to make a dilution in ammonium oxalate or hypotonic saline to lyse the red blood cells if a heavy red blood cell concentration is obscuring the white blood cells. Acetic acid is not advised, as it will cause a mucin clot to form as described above.\(^2\)

The types of cells occurring in synovial fluid are neutrophils, lymphocytes, monocytes, macrophages, synovial lining cells, eosinophils, basophils, Lupus Erythematosus (LE) cells, Reiter cells, and Rheumatoid Arthritis (RA) cells (see Figure 4).\(^1,2\) It is theoretically possible to find malignant cells in joint fluid, but this is rare.

Neutrophils should compose less than 25% of the total differential cells, and lymphocytes less than 15%. The predominant cell type in normal joint fluid should be mononuclear cells, including monocytes, macrophages, and synovial lining cells.\(^1\) Mononuclear cells resemble each other so closely and have so little clinical significance on their own that most laboratories group them together in the same category on the differential. Elevated neutrophils (80% or more) usually indicates septic arthritis (Figure 4), whereas an elevated cell count that consists mostly of lymphocytes indicates non-septic inflammation.\(^2\) In cases of suspected bacterial infection, a Gram stain and culture are the next obvious steps.
Synovial lining cells (Figure 5) resemble mesothelial cells in other fluids, and secrete the viscous
hyaluronic acid into the joints. They can proliferate in a reactive setting just like mesothelial cells in other
fluids. They can also appear in clusters and/or be multinucleated.³ Lupus erythematosus cells may rarely
be found in synovial fluid in rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune
diseases. An LE cell is formed when a neutrophil phagocytizes nuclear material from a dead cell,
developing a smooth, homogeneous, pink inclusion that pushes the nucleus to the periphery of the cell.⁴
Reiter cells are vacuolated macrophages that contain neutrophilic debris or unrecognizable bluish
material.³ Reiter cells are not necessarily diagnostic for Reiter disease, an uncommon type of reactive
arthritis where inflammation affects the eyes and urethra as well as the joints.² Rheumatoid arthritis cells
are also called ragocytes and are neutrophils with small, dark granules of precipitated rheumatoid factor
in the cytoplasm. Despite their name, RA cells are not specific for rheumatoid arthritis as they may also
be seen in gout and septic arthritis.² It is also possible but uncommon to see hemosiderin granules in
clusters of synovial lining cells, indicating a condition called pigmented villonodular synovitis.¹,²
Synovial Crystals

A crystal examination is generally included on a complete synovial examination but can also be ordered as a stand-alone test, as it is one of the most useful and specific diagnostic tests in an arthritis workup. Crystals in the joint fluid cause acute inflammation, producing pain, redness, and heat at the affected joint area. The condition can resolve on its own, depending on the cause, or it can become chronic with repeated acute attacks.5

The three most common types of pathologic crystals seen in synovial fluid are monosodium urate (MSU), calcium pyrophosphate dihydrate (CPPD), and cholesterol crystals.3 Artifacts such as talc or starch from gloves, steroid particles from injection therapy, or plastic shards from a degenerating artificial joint can also be seen and sometimes mistaken for true joint crystals. Even powdered anticoagulants can appear like crystals, which is why it is recommended to collect synovial fluid for crystal examination in a (liquid) heparinized syringe and a plain red-top tube with no anticoagulant.

Crystal Examination

Crystals are examined by using a polarizing light microscope (Figure 6). A fixed light filter, the analyzer, is placed between the specimen and observation tube and a rotating filter, the polarizer, is placed between the specimen and the light source.6 On many microscopes, the analyzer is simply a slide switch under the microscope head. The polarizer could be screwed onto the base, or held there manually.

Both the analyzer and the polarizer allow light to pass in one direction only. When they are rotated 90° to each other, no light can pass, producing the dark field. Any material in the specimen that is birefringent, or able to refract light, changes the direction of the rays and shows bright against the dark field.3

The polarizer kit should also include a quartz compensator that swings into place once the dark field is determined. The compensator separates the light rays into slow and fast vibrations and produces a red background. Based on the different linear structures of the MSU and CPPD crystals, the color of the crystals when aligned with the slow vibration (Z axis) can be used for identification.3 The MSU molecules run parallel to the long axis of the crystal and produce a yellow color when aligned with the Z axis on the
polarizer. This is called negative birefringence. Monosodium urate crystals are strongly birefringent and are long, pointed needle shapes. See Figure 7.

In contrast, CPPD molecules are perpendicular to the long axis of the crystal and appear as blue when aligned with the Z axis (Figure 7). This is called positive birefringence. Calcium pyrophosphate dihydrate crystals are weakly birefringent (not as brightly colored) and more variable in shape. They are usually rhombic or rectangular, but they can also appear as short rods. When the Z axis is reversed, so is the color of the crystal, so care must be taken to note which direction the Z axis of the polarizer is in relation to the crystal in question.

The third type of common synovial crystal is cholesterol (Figure 8). Cholesterol crystals are also strongly birefringent, but variable in color compared with the Z axis. They can be identified by their flat, plate-like shape with unmistakably notched corners. Cholesterol crystals can be found in chronic inflammatory conditions such as rheumatoid arthritis.
Crystal-Induced Inflammation

What is gout or pseudogout, and how do crystals infiltrate the joint fluid? The exact mechanism of crystal formation is not known, but they seem to form in the joint cartilage and are extruded into the joint fluid between the bones. This can cause extreme pain and inflammation when the body reacts to the presence of the foreign crystals.

Gout is a disease that primarily strikes men older than 40 years. Most commonly it first attacks the lower extremities, typically the big toe. It is diagnosed by identifying MSU crystals in the joint, caused by either the overproduction or under-excretion of uric acid. People with chronic gout may also get lumps of MSU crystals in the soft tissue called gout tophi (Figure 9).

Pseudogout is an older term for what is now called CPPD deposition disease. It is predictably triggered by CPPD crystals in the joints. The symptoms are very similar to gout, hence the term pseudogout. CPPD deposition disease usually occurs in men or women older than 60 years but can also afflict a younger person if an injury or other predisposing condition is present.

Summary

Arthritis can be caused by a variety of conditions and can be either acute or chronic. Synovial fluid analysis is used to determine the pathogenicity of the arthritis, whether due to infection, inflammation, trauma, age degeneration, or immunologic disorders. Total cell counts and crystal identification are two
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of the most common diagnostic tests on synovial fluid, as well as Gram stain and culture in the event of infection.

References


For quiz question #3, examine the cell at the end of the arrow: