166 Joint Fluid

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Definition

Joint fluid is a transudate of plasma that is actively secreted by synovial cells. It provides nutrients and lubrication for articular cartilage. Examination of joint fluid focuses on those properties and constituents of value in determining the cause of effusions. A definitive diagnosis from joint fluid findings can be made only in two situations: crystal synovitis and septic arthritis. In other conditions, the correlation of joint fluid analysis with clinical information is useful in arriving at a diagnosis.

Technique

There is no routine for joint fluid analysis. Therefore, it is the responsibility of the examining physician to record a gross description of the fluid (volume, color, clarity, and viscosity). As a minimum, a white blood cell count with differential, a microscopic examination under polarized light for crystals, a gram stain, and bacteriologic cultures should be done. Glucose and protein are ordered only if there is sufficient fluid. Special cultures and chemistries may be indicated in unusual circumstances. The mucin clot test can be done, but adds little to the viscosity, white blood cell count, and differential as a measure of inflammation.

Recent studies have shown a considerable variation in what various laboratories consider to be routine and in the accuracy of their reports. Consequently, it is prudent to specify the desired tests rather than simply request a "routine" analysis. The physician should develop some facility in performing and interpreting a microscopic examination, in the event that the laboratory is not always immediately accessible, or if the laboratory report is questioned. The following materials should be available before an aspiration is performed:

- Betadine solution for skin preparation
- Alcohol swabs to remove iodine from the puncture site
- 1% lidocaine for local anesthesia
- Sterile syringes:
  1 for anesthesia: 5 cc with 25-gauge needle
  1 or 2 for aspiration: 10 or 20 cc with 22- or 20-gauge needles
- Heparin solution for flushing the larger syringes prior to aspiration
- Collecting tubes:
  1 sterile culture tube
  1 fluoride (gray-top) tube for glucose
  1 or 2 plain or lavender-top tubes for blood count and other chemistries
- 2 glass slides
- 1 cover slip
- Band-Aid
- Optional: Clear nail polish for sealing the edges of the cover slip

If 2 ml or more of fluid is obtained, it should be apportioned as follows:

Amount	*Tube*	*Test*
0.5 to 1 ml	Sterile	Culture and gram stain
0.5 to 1 ml	Plain or lavender top	White blood cell count and differential
0.5 to 1 ml	Gray top	Glucose
1 to 2 ml	Plain or lavender top	Protein; special chemistries
0.5 ml	Green top	Crystals

If a smaller amount of fluid is obtained, the first few drops should go into the culture tube. One drop should be spread on one of the glass slides for gram stain, and another drop should be placed on the other slide for crystal examination and covered with a cover slip. The edges may be sealed with clear nail polish if the slide will not be examined immediately. Viscosity can be checked by observing the length of the string formed as the syringe is pulled away from the slide. Normal fluid will form a string 5 to 8 cm in length before breaking. Any remaining fluid may be placed in an appropriate tube and sent for complete blood count, differential, and those tests deemed most useful. Cell counts and crystal examination can be performed on fluid that is 1 or 2 days old, if refrigerated, though there will be some cell loss. Chemistry determinations should be done promptly or else the fluid should be centrifuged and the supernatant refrigerated.

To examine for crystals, the wet mount is placed on a polarizing microscope and the cells or other particulate matter brought into focus under low power. Under high dry magnification, an estimate of the cell count and the proportion of polymorphonuclear cells (PMN) can be ascertained. When the light is reduced by the condenser, it is often possible to see crystals, if present. The polarizer or analyzer disc is then turned until the field becomes dark. Birefringent crystals appear light against the dark background. Although monosodium urate crystals are typically long and needle shaped, one should not rely on morphology for identification. Moving the red compensator into the light path will produce a blue or yellow color to birefringent particles. A reference arrow inscribed on either the analyzer or the compensator plate allows identification of crystals. A common convention among house officers is the word *BRAG;* if a *B*lue crystal is at *R*ight *A*ngles to the reference arrow, *G*out is likely (the crystal is monosodium urate). If the slide can be rotated 90 degrees, or if another urate crystal can be found parallel to the reference arrow, the crystal will be yellow. Calcium pyrophosphate crystals will give the opposite color changes (yellow when perpendicular to the ar-

row, and blue when parallel). The color intensity of the calcium crystals is less than that of urate crystals. Gatter (1984) contains excellent color plates of crystals and other particles and cells that can be identified microscopically. If a polarizing microscope is not available but crystals can be seen, a drop of 0.1 *N* NaOH can be added to the slide. Urate crystals will dissolve. A drop of freshly filtered 2% Alizarin Red S can be added to a drop of fluid on another slide; calcium crystals of any type will then appear orange under the microscope.

If heparin is not used in the aspirating syringe, a green-top tube (sodium heparin) is suitable for crystal analysis. Tubes containing lithium heparin or oxalate as anticoagulants should not be used, since crystals resembling those of urate or calcium may form. Iodine from the skin prep may also introduce crystals if not removed from the puncture sites. An anticoagulant is necessary, since inflammatory fluid may clot and yield a falsely low white blood cell count. Saline (0.3%) should be used to dilute the fluid for the white blood cell count because the standard acetic acid diluting fluid will cause the fluid to clot. Lidocaine may suppress bacterial growth; therefore, the anesthetizing syringe or needle should not be used for aspiration.

Monosodium urate crystals show intense negative birefringence. Betamethasone crystals are also negatively birefringent and can be used as a standard reference slide by the novice. Unfortunately, if this steroid was recently injected into the aspirated joint, it may confuse interpretation. Calcium salts, including the oxalate used as an anticoagulant in collecting tubes, are positively birefringent, as are lithium heparin and some steroid crystals. Artifacts such as dust, talc, and nail polish show variable birefringence. Calcium hydroxyapatite crystals are tiny and therefore difficult to identify on a wet mount; however, clumps will stain purple with Wright's stain and orange with Alizarin Red S.

Basic Science

Synovial fluid is secreted by synovial cells lining the joint capsule, but not the articular cartilage. The synovial lining is not continuous and lacks a basement membrane. The subsynovial layer contains lymphatics and blood vessels supported by a fibrous joint capsule. Therefore, a few erythocytes are usually seen after arthrocentesis, secondary to either capillary trauma or the underlying disease. Selected components of the synovial fluid usually undergo predictable changes depending on pathophysiologic processes (Table 166.1).

Joint fluid in normal and noninflammatory conditions is transparent, that is, fine print can usually be read through a test tube of fluid. Clarity changes from transparent to opaque in direct proportion to the number of leukocytes that enter the fluid through inflammation or infection. With minimal inflammation, capillary leakage of red cells, and the metabolism of hemoglobin to bilirubin, the fluid may become xanthochromic. Color is also affected by the presence of leukocytes and bacteria, with pus being off-white. For example, *Staphylococcus aureus* may produce a golden hue. A bloody red fluid may be due to trauma or other causes of hemarthrosis. Less than 10% of blood can cause the joint fluid to look like whole blood; hence, a simultaneous serum and synovial hematocrit may be necessary to determine the amount of blood present.

Joint fluid in normal and noninflammatory conditions contains a significant amount (300 mg/dl) of hyaluronic acid, a high-molecular-weight polymerized glycosaminoglycan, which is responsible for the unique eggwhite-like consistency and high viscosity of joint fluid. In inflammatory or combined inflammatory–infectious processes, hyaluronate may be fragmented by the proteolytic action of lysozymes released by polymorphonuclear cells (PMNs). This results in a decreased concentration and a low viscosity.

Joint fluid in normal and noninflammatory conditions contains white blood cells, predominantly monocytes, lymphocytes, macrophages, and a few PMNs. In inflammatory and infectious conditions other than tuberculosis or viral disease, the fraction of PMNs increases in proportion to the increases in total synovial fluid leukocyte count; and the total leukocyte count increases in proportion to the extent of inflammation and infection. Consequently, the major basis for classifying a synovial effusion is quantification of leukocytes (Table 166.1). Leukocyte counts vary over a wide range with some overlap between groups due to the variability of the mechanisms producing the joint fluid (Table 166.2). Even for a specific disease such as rheumatoid arthritis, the leukocyte count can vary from 2000 to 75,000/mm^3 depending on the activity of the joint disease. Combined pathophysiologic processes may make interpretation of the leukocyte count difficult. For example, patients with a noninflammatory process such as hemochromatosis may have significantly higher than expected leukocyte counts if an associated inflammatory process such as a crystal-induced arthritis is also present.

Table 166.1
Joint Fluid Classification According to Test Results

	Normal	Group I (noninflammatory)	Group II (inflammatory)	Group III (infectious)	Group IV (hemorrhagic)
Appearance	Transparent	Transparent	Translucent or opaque	Opaque; not clear	Opaque
	Clear or pale yellow	Yellow	Yellow	Creamy; green	Red
Viscosity	High	High	Low	Variable	Variable
WBC/mm^3	<200	150–3000	2000–75,000	50,000–300,000	...[a]
PMN (%)	<25	<25	50–100	75–100	...[a]
Glucose (% of blood level)	90–100	90–100	40–90	≤50	90–100

[a]The WBC and PMN percentages will be determined by the proportion of free blood in the joint fluid. It may be difficult to rule out a coexisting inflammatory process.

Table 166.2
Differential Diagnosis of Joint Fluid Groups

Group I (noninflammatory)	Group II (inflammatory)	Group III (septic)	Group IV (hemorrhagic)
Osteoarthritis	Rheumatoid arthritis	Bacterial infection	Trauma
Trauma	Crystal synovitis	Rheumatoid arthritis	Coagulation defect
Avascular necrosis	Spondyloarthropathy	Crystal synovitis	Tumor
Systemic lupus	Reactive arthritis		Pigmented villonodular synovitis
Acute rheumatic fever	Connective tissue disorders		
Endocrine arthropathy	Acute rheumatic fever		
	Juvenile arthritis		
	Sarcoidosis		
	Tuberculosis		
	Viral infection		
	Fungal infection		
	Bacterial infection		

Joint fluid in normal or noninflammatory conditions does not usually contain crystals, unless the crystal-induced arthritis is in a quiescent state, or unless nonpathogenetic crystals such as cholesterol or artifacts such as previously injected corticosteroid esters are present. The two predominant crystals that can produce synovial inflammation are monosodium urate monohydrate (MSU) in gout and calcium pyrophosphate dihydrate (CPPD) in pseudogout. Although the fundamental mechanism of crystal-induced formation in synovial fluid is unclear, the association of crystals with the inflammatory response is well documented. For example, it is rare to find crystals without neutrophils or neutrophils without crystals during acute episodes. Less frequently seen are monocytes and synovial cells phagocytizing these crystals. Whether intracellular or extracellular, these crystals appear needlelike (MSU) or rhomboid (CPPD); yet both may appear as blunt rods.

Joint fluid in normal and noninflammatory conditions usually maintains a glucose level close (within 10 mg/dl) to the serum concentration because glucose enters the synovia from blood by facilitated diffusion with equilibration. The synovial–serum difference is most reliable only in the fasting state because equilibration is slow and unpredictable after a meal. In inflammatory and infectious conditions, synovial glucose is often reduced as a result of its utilization by the metabolic activity of the neutrophils and bacteria.

Joint fluid in normal and noninflammatory conditions contains about one-fourth the total protein present in blood. Coagulation proteins are absent, hence normal joint fluid does not clot. Smaller molecules, such as albumin, are usually present in greater concentrations than larger molecules, such as most of the globulins. In inflammatory conditions, however, the concentration of these components may be equal to the plasma concentrations because of increased synovial blood flow. Synovial fluid protein levels greater than 2.5 g/dl are abnormal, and those greater than 4.5 g/dl indicate significant inflammation.

In inflammatory effusions, a gram stain and culture are mandatory to rule out a septic joint as well as infection superimposed on another condition, such as rheumatoid arthritis. The lack of a basement membrane makes the synovium a susceptible site for infection in the presence of bacteremia.

Components of the immune system, such as antinuclear antibodies, IgG and IgM rheumatoid factors, and total hemolytic complement, usually parallel changes in the serum and/or are nonspecific. Other nonspecific synovial fluid measurements and their associated processes include a decreased pH (inflammation), increased lactic acid (nongonococcal septic arthritis), and lysozyme/lactoferrin ratio (extent of inflammation), to name a few. None of these tests, however, should be routinely ordered on synovial fluid.

Clinical Significance

Analysis of joint fluid yields a definitive diagnosis in only two situations: septic arthritis and crystal-induced synovitis.

Septic arthritis is an emergency because a pyogenic infection can destroy a joint if appropriate therapy is not initiated within days. Purulent fluid (group III) mandates antibiotic therapy even if the gram stain is negative. Synovial fluid gram stains are positive in less than 25% of cases of gonococcal arthritis, and a positive synovial fluid culture is obtained in only 50% of cases. The gram stain is positive in 50 to 75% of cases of gram-negative bacillary and staphylococcal infections, respectively, but cultures are almost always positive. Cultures may be spuriously negative if the synovial fluid contains lidocaine or if the patient has received earlier antibiotic therapy. Gram stain yield is improved if a centrifuged sediment is obtained.

Group II fluid is sometimes obtained in septic arthritis. This may occur if the fluid is removed early in the course of the disease, if the patient is taking antibiotics, if the patient is immunocompromised, or if the fluid is taken from an adjacent bursa rather than the joint space. Indolent effusions with minimal discomfort and inflammation suggest a tuberculous or fungal cause. Appropriate cultures and/or synovial biopsy are indicated in such cases.

The presence of monosodium urate or calcium crystals in an inflammatory fluid, especially when the crystals are intracellular, warrants a diagnosis of *crystal-induced synovitis*. Crystal synovitis, however, does not exclude a concomitant infection. Therefore, gram stain and culture must still be done. Furthermore, calcium and urate crystals may be present in the same joint fluid; for example, a patient with chondrocalcinosis may have gout, or a patient with gout may have secondary calcium deposits in an adjacent tophus. Artifacts from the use of the wrong anticoagulant or from prior steroid injections may be misleading (see Technique). Calcium pyrophosphate is the crystal associated with the synovitis of chondrocalcinosis (pseudogout). Chondrocalcinosis may be hereditary or related to osteoarthritis, hyperparathyroidism, hemochromatosis, hypothyroidism, gout,

acromegaly, ochronosis, hypophosphatasia, or hypomagnesemia. Calcium salts other than pyrophosphate have been associated with synovitis. Hydroxyapatite crystals have been associated with both osteoarthritis and an erosive arthropathy. Calcium oxalate crystals have been found in synovial effusions of patients undergoing hemodialysis.

In the presence of bacterial infection, synovial fluid glucose may be at least 25 mg/dl lower than a simultaneous blood glucose, providing the patient is fasting. Rheumatoid effusions may also show a low glucose. Synovial fluid protein rises proportional to the degree of synovial inflammation. This rise is nonspecific and adds no clinically useful information beyond that obtained from the white cell count and differential. Synovial fluid lactic acid levels have been suggested as a quick method of identifying a septic joint. Lactic acid levels appear to be proportional to the white blood cell count, however, and therefore have no greater specificity than the white blood cell count.

Synovial fluid viscosity falls in proportion to the degree of inflammation, so that a very low viscosity of fluid with a low white blood cell count suggests a technical error in performing the cell count. This can occur with a long delay before performing the cell count, or clotting of the fluid. Extremely viscous fluid may be found in the myxedematous patient.

In conclusion, valuable diagnostic information can be obtained from analysis of joint fluid. When standard aseptic technique is employed, the likelihood of introducing infection is rare. (Sterile gloves and mask are not absolutely necessary.) Therefore, a physician should not hesitate to obtain joint fluid for analysis when evaluating an unexplained joint effusion. Joint fluid analysis is essential for the evaluation of a patient with acute monoarthritis.

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