Preanalytical Considerations for Joint Fluid Evaluation

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KEYWORDS

• Synovial fluid • Orthopedics • Osteoarthritis • Immune-mediated polyarthropathy
• Septic arthritis

KEY POINTS

- Synovial fluid analysis is a key component of the minimum database needed to diagnose and manage primary and secondary articular joint disorders.
- Preanalytical variables can drastically alter samples submitted to veterinary laboratories for evaluation and it is considered the stage at which most laboratory error occurs.
- Common indications for the collection of synovial fluid include orthopedic lameness; abnormal limb function or gait; articular pain and/or distention; fever of unknown origin; or widespread pain, stiffness, or difficulty moving.
- The gross characteristics of synovial fluid yield important information as to the quality and pathology of the joint and should be noted at the time of sample collection.
- When arthrocentesis yields small sample volumes, cytologic evaluation of direct smears offers the most clinically relevant information available from synovial fluid.

INTRODUCTION

In concert with clinical history, physical examination, imaging modalities, and other clinical pathology findings, synovial fluid analysis is a key component of the minimum database needed to diagnose and manage primary and secondary articular joint disorders. The preanalytical phase of synovial fluid evaluation encompasses the steps from initial sample collection through final arrival and accessioning at the diagnostic laboratory. Unfortunately, preanalytical variables can drastically alter samples submitted for evaluation to veterinary laboratories and it is considered the stage at which most laboratory error occurs.[1] Specific variables that may influence the interpretation

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of joint fluid mostly involve technical factors, such as selection of collection materials, evidence of blood contamination, and specimen handling. Other considerations that should be included with sample submission include the location that was aspirated, the signalment and presenting signs of the animal, and current medications being administered. With consistent preanalytical quality control and reporting of specimens, downstream clinical decision making and management of patients can be accelerated and improved. General practitioners should always feel comfortable contacting a diagnostic laboratory for specific information regarding sample acquisition, processing, and submission.[2,3]

ORIGINS AND CHARACTERISTICS OF SYNOVIAL FLUID

Joints are the points of interaction and movement between 2 adjacent bones. Hyaline cartilage overlays the subchondral bone and is the contact surface. Chondrocytes within the articular cartilage produce the proteoglycans present in the cartilage. This articular space is enclosed by a joint capsule. The joint capsule is composed of a tough, fibrous external surface, a vascular subsynovial layer, and the lining synovial surface. The synovium is thin and contains 2 types of cells, or synoviocytes. These cells are either phagocytic and thus similar to macrophages (type A synoviocytes), or they produce hyaluronic acid, a glycosaminoglycan (type B synoviocytes).[4] Both types of synoviocytes can be visualized on cytologic preparations of synovial fluid, but are typically difficult to differentiate in normal joints and are classified together as large mononuclear cells.

Synovial fluid from a nondiseased joint is an ultrafiltrate of plasma that is modified by secretion of hyaluronic acid, glyocoproteins, and other macromolecules. Smaller molecules, such as glucose and electrolytes, can occur in synovial fluid in concentrations equivalent to those in plasma. Proteins from plasma, however, are included in this synovial dialysate only to a limited extent. Synovial fluid serves 2 major purposes. First, it provides a source of nutrition and waste removal from articular chondrocytes. Second, joint fluid lubricates opposing articular cartilage surfaces, limiting friction and wear during contact. Although hyaluronic acid has some lubricating properties, glycoproteins are the primary source of reduced friction during joint motion.

When the components of the joint become diseased, injured, and/or inflamed, pathology is frequently reflected by changes within synovial fluid. Evidence of abnormal conditions include joint effusion with decreased viscosity and increased protein content, increased numbers of inflammatory cells, changes in the percentages of different cell types present, and intra-articular hemorrhage.

INDICATIONS FOR COLLECTION OF SYNOVIAL FLUID

Synovial fluid analysis is a critical aspect of the workup for an animal exhibiting joint issues, whether these concerns are primary maladies localized to one or more limbs or reflect the secondary manifestation of a systemic condition. Importantly, these data must be integrated with other clinical and laboratory findings, including culture, serology, antinuclear antibody titer, and rheumatoid factor titer. Common indications for the collection of synovial fluid include orthopedic lameness, abnormal limb function or gait, articular pain and/or distention; fever of unknown origin; or widespread pain, stiffness, or difficulty moving.[5]

Typical characteristics of synovial fluid from dogs and cats are presented in **Table 1**. Normal synovial fluid is light yellow and clear, with no observable particular material. The volume that can be aspirated depends on the joint being sampled and the condition of the joint. For most large breed dogs, less than 0.5 mL of synovial fluid is

Table 1
Normal synovial fluid characteristics in dogs and cats

Species	TNCC (Cells/μL)	% Neutrophils	Expected Volume, mL	Gross Appearance
Dog	≤2900	≤12	≤0.5	Light yellow
Cat	≤1134	≤39	≤0.25	Clear Highly viscous Thixotropic

Abbreviation: TNCC, total nucleated cell count.
Data from Refs.[11,18,19,37]

expected from most joints. Up to 1 mL of fluid can be collected from stifle joints,[6] and the volume of joint fluid obtained from dogs with hip dysplasia can range from 2 to 6 mL.[7] Joints from small dogs and cats do not typically yield more than 0.25 mL of synovial fluid.

RECOMMENDATIONS FOR SAMPLE COLLECTION

Collection Sites

Routine sterile technique should be followed for all arthrocentesis procedures, including the clipping of fur and thorough scrubbing of the area. The clinician should judge the amount of restraint and sedation or anesthesia needed to accomplish joint fluid collection in the absence of complications. Ultrasonography is frequently used and recommended for guiding arthrocentesis procedures and characterizing joint lesions in humans.[8,9] Unwanted events include blood contamination, retrieving an insufficient volume of synovial fluid, scratching articular surfaces, or damaging local blood vessels, nerves, or the synovial membrane. Before removing the needle from the joint, the plunger of the syringe should be released to eliminate negative pressure.

Brief descriptions of recommended approaches to synovial fluid collection from articular joints are provided in **Table 2**. When noninfectious polyarthritis is a differential diagnosis, it is recommended that arthrocentesis be performed on multiple joints, specifically the carpi, tarsi, and stifle joints. Clinical signs commonly associated with polyarthritis include leg-shifting lameness, fever of unknown origin, and joint pain. Of note, however, one retrospective study documented that polyarthritis also can present simply as difficulty walking, without any other significant findings.[10]

Collection Materials

Needles and syringes

Sterile disposable 0.75-inch to 1-inch, 22-gauge or 25-gauge hypodermic needles connected to 3-mL to 6-mL syringes are recommended. Some researchers prefer a 25-gauge needle attached to a 1-mL or 3-mL syringe for feline arthrocentesis, as increased blood contamination is noted with larger-bore needles.[11] In larger-breed dogs, sampling of the shoulder or elbow joint may necessitate a 1.5-inch needle, whereas the hip may require a 3.0-inch spinal needle.

Tube selection

When inflamed synovial membranes have increased vascular permeability, this allows increased proteins, such as fibrinogen, into joint fluid. This often results in clotting of synovial fluid samples. Clotting can also occur with significant hemorrhage or blood contamination. Given this, it is recommended to aliquot a portion of the sample into an ethylenediaminetetraacetic acid (EDTA) tube to prevent coagulation and allow for additional analyses.[5] The smallest EDTA blood tube available should be used to avoid

Table 2
Suggested positioning and entry for arthrocentesis of common joints

Articular Joint	Position	Needle Entry	Needle Advancement
Stifle	Flexed	Lateral to patellar tendon and distal to patella	Needle is advanced in a medial and proximal direction, pointing toward the medial condyle of the femur
Hip	Femur is abducted and leg extended caudally	Cranial to the greater trochanter of the femur	Needle is inserted caudal and distal to the joint
Shoulder	Neutral to flexed	Distal to the acromion of the scapula and caudal to the greater tubercles of the humerus	Needle is directed medially toward the greater tubercle and just distal to the supraglenoid tubercle of the scapula
Elbow	Extended or flexed	Extended: Medial to the lateral epicondyle of the humerus and lateral to the olecranon Flexed: Proximal to the olecranon and medial to the lateral epicondylar crest	Needle is inserted parallel to the olecranon and the long axis of the ulna
Carpus	Flexed	Antebrachiocarpal joint: Between the distal radius and proximal radial carpal bone Middle carpal joint: Distal portion of the radial carpal bone and the second and third carpal bones	Needle is introduced from the dorsal aspect, just medial of center, and inserted perpendicular to joint
Tarsus	Extended or flexed	Extended (caudal approach): Medial or lateral to the calcaneus Flexed (cranial approach): Space palpated between tibia and talus bones, just lateral to the tendon bundle	Extended: Needle is advanced with a cranial and slightly plantar path Flexed: Needle is inserted perpendicular to palpated space

erroneous results due to sample dilution from excess anticoagulant. If small tubes are not available, then excess EDTA can be decanted from the vessel before adding the sample.

In contrast, EDTA can cause disruption of hyaluronic acid. Therefore, it is not recommended to evaluate synovial fluid viscosity or mucin clot formation from EDTA samples. When this is desired, sample should be provided in a tube that does not contain this particular anticoagulant, such as a red top tube or a heparin tube.[12]

For samples that are to be cultured, fluid should be left in the sterile syringe and/or placed into an aerobic culturette or blood culture tube. A red top tube also may be used for this purpose if sterility can be maintained. To increase the likelihood of a positive culture, it is recommended to place the synovial fluid sample in blood culture media if available. Synovial membrane biopsy for culture is not recommended, as it has been shown to be less successful at yielding positive culture results than synovial fluid culture. Additionally, synovial membrane biopsy is considerably more invasive and traumatic than arthrocentesis.[13]

Sequential Arthrocentesis

Sequential arthrocentesis and synovial fluid analysis is often performed as part of the therapeutic monitoring of immune-mediated polyarthritis. It is recognized that arthrocentesis is a traumatic procedure and may elicit mild mononuclear inflammation. However, one study performed in healthy dogs showed that synovial fluid collection every 3 weeks did not result in differing cell counts or percentages.[14] Thus, repeated collections using this length of time are considered less likely to be clinically significant or obscure evidence of a therapeutic response.

VISUAL ASSESSMENT OF COLLECTED SAMPLE

The gross characteristics of synovial fluid yield important information as to the quality and pathology of the joint and should be noted at the time of sample collection. Even when limited sample is available and only a direct smear can be prepared, it is still possible to evaluate the approximate volume, color, viscosity, and turbidity of synovial fluid as the sample is aspirated, expelled onto a slide, and smeared.[15] Homogeneously red or red-tinged fluid indicates hemorrhage in the joint, which is usually associated with trauma or inflammation. This should be differentiated from blood contamination, as discussed later in this article. Xanthochromia, which imparts a yellow-orange discoloration to joint fluid due to hemoglobin breakdown, may be seen following the resolution of hemorrhage. Overt sediment and/or white or light yellow coloration indicates either an increase in the nucleated cell count, usually due to inflammation, infection, or neoplasia, or the presence of crystals. Increased turbidity results from suspended particulate matter, which may be related to red or white cells, fibrin, bacteria/organisms, or, less commonly, crystals. It is rare for crystals or neoplastic cells, alone, to cause changes in color or turbidity.[16]

It is important to recognize that normal synovial fluid does not clot in a tube or syringe, but instead forms a gel. Distinction of these 2 states can be accomplished by agitation of the sample, which will cause the gel, but not a clot, to return to its fluid state. This reversible fluid-gel phenomenon is called thixotropism.

VALUE OF DIRECT SMEAR PREPARATION

Small animal arthrocentesis often yields limited sample volume. In such scenarios, it is commonly advised that cytologic evaluation of direct smears offers the most clinically relevant information available from synovial fluid.[15,17,18] Given the viscosity of joint fluid, a squash or compression preparation is typically used to generate direct smears. It is recommended that direct smears be made immediately following sample collection and before any additional processing. This prevents artifacts or misinterpretations due to sample aging, concentration, dilution, or contamination.[2,3] A rough estimation of total nucleated cell count (TNCC), differential counts, characterization of any unusual cells that may be present, as well as a subjective assessment of the proteoglycan background, are all possible to evaluate from a direct smear, alone. Indeed, direct smears are often preferred over cytocentrifuge-prepared slides for differential cell counts, as some researchers feel that the latter may not provide an accurate representation of cell proportions and that the morphology of the cells may be altered during centrifugation.[2,19] In particular, a diagnosis of suppurative inflammation can be reliably made using direct smears.[20] If sample quantity allows, recommended additional tests include TNCC (obtained via automated or hemocytometer methods), total protein, and mucin clot test. If there is concern for bacterial arthritis, 2 to 3 drops of synovial fluid for culture and sensitivity can be placed into a culturette.

One issue with direct smears is that highly viscous synovial fluid often yields thick preparations with cell clumping and severely rounded up cells that cannot be easily identified or counted. This tends not to be a problem, however, when the joint is diseased and the synovial fluid is dilute. To address thick samples, hyaluronidase can be added to synovial fluid before slide preparation. It is recommended that 150 IU/mL hyaluronidase be mixed with an equal volume of joint fluid and incubated for several minutes before direct smear slide preparation.[19] When used, it is suggested to use gentle smearing technique, as the hyaluronidase treatment tends to cause the cells to become more fragile.

Direct smears should always be evaluated before submission to ensure adequate cellularity and distribution of synovial fluid. However, certain caveats should be emphasized, even to pathologists. First, it has been shown that the estimation of total cell count on direct smears varies from clinician to clinician and can often overestimate true cell numbers.[20] Further, distinguishing between normal synovial fluid and synovial fluid reflective of degenerative joint disease or osteoarthritis is not consistent using direct smears. Finally, direct smears should not be used as the sole indication of disease progression or evidence for therapeutic efficacy.[20]

KEY INFORMATION TO PROVIDE WITH SAMPLE SUBMISSION

Signalment

The species, age, breed, and gender of an animal can provide key information for prioritizing potential diagnoses. For example, synovial cell sarcoma is a rare tumor in animals, but occurs far more commonly in dogs than cats.[21] Clinical signs of polyarthritis syndrome in akitas typically appear before 8 months of age.[22] Bernese mountain dogs have the highest prevalence for the systemic form of histiocytic sarcoma; however, skeletal manifestation of this disease occur far more commonly in rottweilers and golden retrievers.[23]

Pertinent Clinical History

Patient history can help characterize the disease process and narrow the differential list. For example, bacterial septic arthritis may develop following articular surgery, local trauma and direct inoculation, extension from regional infections, or hematogenous spread.[24] In one case, septic arthritis occurred secondary to a migrating porcupine quill, which had been reported to travel 10 inches under the skin.[25] Bacterial septic arthritis is often thought to present as acute, severe lameness but it may also develop as chronic, mild pain without significant orthopedic impairment.[26] Immune-mediated polyarthropathy has been associated with a variety of processes, such as vaccine reactions, drug reactions, existing neoplasia, gastrointestinal disease, and idiopathic causes.[27] Suspicion for foreign body reactions, with or without secondary infection, also relies on a complete discussion of pertinent events, even if these events are not recent. For instance, onset of severe arthropathy was reported in a dog 21 months after being shot in the elbow with a lead bullet.[28] Interestingly, the metallic fragments did not mechanically interfere with joint motion.

Location(s) Aspirated

Noting the joints affected and the joints aspirated can help delineate the full extent of disease and place emphasis on one diagnosis over another. For instance, the inflammation associated with immune-mediated polyarthropathy is thought to be elicited by complement activation following antigen-antibody complex deposition within the

synovium of multiple joints. Of note, lesions are most commonly observed in smaller joints, such as the carpi and tarsi.[29]

Current Medications and Nutritional Supplements

Administration of any medication should be provided on the submission form, especially if that treatment is directed at a disorder on the differential list. This includes systemic medications, as well as local, regional, and topical drugs. Of note, it is well-demonstrated that the application of nonsteroidal anti-inflammatory drugs on the skin can penetrate local tissues into the synovial fluid.[30]

It also may be pertinent to disclose the use of nutraceuticals and other supplements. Clinical trials that support the use of glucosamine, chondroitin sulfate, P54FP (a turmeric extract), omega-3 fatty acids, *Boswellia serrata* (a tree extract), and avocado-soybean unsaponifiable extracts in dogs with osteoarthritis, after surgery or trauma, and for prophylactic measures have been reported.[31] However, these studies are limited in number and there remains a need for additional randomized controlled clinical trials. Further, despite the perception that joint health products are safe based on high LD50 values and an absence of persistent adverse effects, veterinarians should be aware that contamination issues, including toxins, pesticides, and heavy metals, and metabolic concerns, particularly the use of glucosamine in individuals with type 2 diabetes, are being addressed in human and animal studies.[31]

Blood Contamination

Careful observation during arthrocentesis may demonstrate a flash of blood in the sample, indicative of blood contamination. Alternatively, a diffusely bloody sample may indicate that the sample is associated with true hemorrhage. As these separate scenarios may appear similar following shipment and arrival to the laboratory, it is important that differentiation of the 2 be documented during sample submission.[5]

SHIPPING RECOMMENDATIONS

Synovial fluid supplied in tubes or syringes can be stored or shipped at 4°C for up to 24 hours without significant loss of cytologic integrity. Therefore, it is recommended that samples being sent to laboratories be packaged with cold packs and shipped overnight to maintain sample value.[32] When providing direct smears, ensure that these slides are completely dry before packaging and kept away from exposure to formalin fumes. Do not refrigerate slides, as subsequent condensation may result in cell lysis.

DIFFERENTIAL DIAGNOSES

Several excellent textbook and review articles are available that discuss approaches to the analytical phase of synovial fluid evaluation. This stage includes cytologic evaluation of the sample, as well as a description of its appearance, protein content, viscosity, mucin clot test, nucleated cell count, and differential. In particular, the reader is referred to a wonderfully thorough article by MacWilliams and Fredericks, published by this compendium in 2003.[16]

Briefly, during the analytical phase of cytologic examination, cells will be differentially counted as small mononuclear, large mononuclear, or neutrophils. Cells will be further characterized by nuclear morphology (such as degeneration in neutrophils) and degree of vacuolization (an indication of activation in macrophages), as well as any other significant features.[29] Other cells that may be present will be described and interpreted. The sample will be carefully screened for the presence of any infectious agents. Cells are typically found on an eosinophilic, finely granular background

Table 3
Three general categories of arthropathies in veterinary medicine and associated differential diagnoses

Hemarthrosis	Noninflammatory Arthropathy
Red to xanthochromic appearance with increased protein, decreased viscosity, and normal to poor mucin clot. TNCC will be increased in proportion to the amount of blood. May see hemosiderin, hematoidin, and erythrophagia.[19,29,38]	Clear appearance with normal to decreased protein and viscosity, normal to poor mucin clot. TNCC will be normal to moderately increased with ≥90% small and large mononuclear cells. Cells may have increased vacuolization.[29,38]
Coagulopathy	Degenerative joint disease
Joint trauma	Joint trauma (ie, cranial cruciate ligament rupture)
Some neoplasms	Some neoplasms

Inflammatory Arthropathy

Cloudy appearance with normal to increased protein, normal to decreased viscosity, and fair to poor mucin clot. TNCC will be mildly to markedly increased with >10%–100% neutrophils.[38] Some specific processes may be predominantly mononuclear or eosinophilic inflammation rather than suppurative.

Infectious	*Erosive*	*Nonerosive*	*Others*
May see degenerate neutrophils. May see infectious organisms. Monoarticular or polyarticular.	Radiographic evidence of articular erosion. Frequently polyarticular.	Erosive and infectious disease have been ruled out. Frequently polyarticular.	May see neoplastic cells or crystals on cytology. Frequently induce an inflammatory response.
Bacteria (cocci, rods, L-forms)[24–26,39] Fungi (*Coccidioides, Aspergillus, Histoplasma, Blastomyces, Cryptococcus*)[40–44] *Rickettsia/Ehrlichia* spp.[34–36] *Borrelia burgdorferi*[27,45] *Mycoplasma* spp[46,47] *Leishmania* spp[48] West Nile virus (dog)[49] Feline calcivirus[50,51] Feline coronavirus (FIP)[52] Canine distemper virus[53]	Rheumatoid arthritis[54] Greyhound erosive polyarthritis[55] Juvenile-onset polyarthritis syndrome in akitas[22] Shar pei fever[56] Chronic progressive polyarthritis (cat)[57,58]	Systemic lupus erythematosus[27] Idiopathic polyarthritis[27] Idiopathic localized eosinophilic arthritis (cat)[59] Drug-induced polyarthritis[60] Lymphoplasmacytic synovitis[61] Postvaccinal arthritis[27,62] Polyarthritis polymyositis syndrome[63]	Joint trauma[28] Neoplasia Primary Histiocytic sarcoma[23,64–67] Synovial cell sarcoma[21,67,68] Synovial myxoma[67] Malignant fibrous histiocytoma[67] Fibrosarcoma[67] Chondrosarcoma[67] Undifferentiated sarcoma[67] Benign giant cell tumor of the tendon sheath[69] Lymphoma[70] Osteosarcoma[71] Hemangioma[72] Metastatic Transitional cell carcinoma[73] Mammary adenocarcinoma[74] Bronchiolar-alveolar carcinoma[75] Crystal-induced arthritis Pseudogout[76]

composed of synovial fluid proteoglycans. Changes in this proteoglycan milieu, such as can occur with dilution, also will be noted and described.

It is important to remember that synovial fluid evaluation cannot be performed in isolation from other diagnostic tests. For example, a presumptive diagnosis of systemic lupus erythematosus requires more than cytologic observation of lupus erythematosus cells and ragocytes. Clinical signs, antinuclear antibody titers, and response to treatment also must be taken into account.[33] In addition, if rickettsial infection is suspected, it is recommended to perform both acute and convalescent serologic titers and polymerase chain reaction tests, as all tests can produce false negatives depending on the specific etiologic agent and stage of infection. Rarely, morulae can be observed in synovial fluid samples but specific identification of the rickettsial agent cannot be confirmed on cytology alone.[34–36] **Table 3** provides a summary of various arthropathies encountered in veterinary medicine.

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