How to perform an arthrocentesis & bone marrow aspiration

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PART I: How to perform an arthrocentesis

Why is performing a joint tap useful?
Performing a joint tap is the single most useful test to distinguish non-inflammatory from inflammatory joint disease. This procedure helps to confirm a diagnosis of degenerative osteoarthritis, septic arthritis, immune-mediated arthropathies and neoplasia.

What is needed to perform an arthrocentesis?
Following material is needed to perform a joint tap:
• Material for surgical site preparation (clippers, swabs, povidone-iodine or chlorhexidine solution, ethanol),
• Sterile gloves,
• 21-25 gauge needles or spinal needles,
• 2-5 ml syringes,
• Pre-cleaned slides and
• EDTA and plain vials.
The use of blood culture bottles should be considered, particular in cases where a slow or scarce growth of bacteria is suspected (e.g. with previous antibiotic therapy, with slow growing bacteria). The correct use of these bottles is described below.

Arthrocentesis in Principle
The animal should be restrained appropriately before the procedure. This may be achieved by sedation, a short general anaesthetic or, at least, a local anaesthetic (under the skin, not into the joint!). The animal should lie comfortably in the appropriate position and the limb from which the joint fluid is to be obtained should not move during the procedure once the proper limb position is achieved. The arthrocentesis site should be clipped and prepared aseptically with a chlorhexidine or povidone-iodine preparation and alcohol. It is important to note that, depending on size of the respective animal and chosen joint, a yield of less than 1 ml joint fluid is common in non-effused joints. In small animals and small (non-effused) joints, total yield may be less than 0.1 ml!
Which site to sample?

The clinical and orthopaedic examination will determine which joint will be the most appropriate to sample from. If several joints are effused, several joints should be sampled. This increases the likelihood that 2 concurrent disease processes (e.g. neoplasia and polyarthritis) are diagnosed and that adequate sample volumes are obtained for analysis. In some cases, particular animals with pyrexia of unknown origin, synovial changes may be present in the absence of effusions. In these cases arthrocentesis of several joints should be performed to establish whether immune-mediated polyarthritis is present. If the client has noted joint swelling, it may be useful to sample the joint that started to swell first - as well as other joints that are affected. The shoulder and stifle joints may obtain the largest quantities of joint fluid in animals that do show no or minimal joint effusions. These joints have the largest cavities. The more distal joints (i.e. carpal, tarsal joints) are easier to palpate, because less muscle mass is present. Thus, it may be easier to determine effusions and where to introduce the needle. In general, the procedure should be completed in an aseptical (surgical) manner, using sterile gloves and sterile needles and syringes. The needle is introduced into the joint with a sterile syringe attached. Negative pressure is applied once the needle has entered the joint. Any negative pressure is released before the syringe is withdrawn.

Performing a joint tap of

1. Carpal joints

The carpus has 3 articulations: the antebrachiocarpal-, middle carpal- and carpometacarpal joint. The 2 distal joints communicate. The most proximal joint (the antebrachiocarpal joint) does not communicate with the distal joints, has the greatest range of motion and is easiest to tap. To tap the joints, partially flex the carpus and palpate the joint spaces. Insert the needle at the dorsal border and direct the needle medially and distally. Take care not to damage the collateral radial artery, accessory cephalic vein and superficial radial nerve, which run dorsally over the carpus and are usually easy to palpate.

2. Elbow joint

To tap the elbow joint, flex the elbow at an angle of approximately 45°. Palpate the olecranon of the ulna and the condyle of the humerus. Check whether effusion can be felt. If effusion is present, insert the needle there. If no is present, insert the needle parallel and palmar to the olecranon and medial to the lateral condyle of the humerus. Advance the needle in craniodistal direction.

3. Shoulder joint

To tap the shoulder joint, flex the joint approximately 30° and rotate the humerus slightly outwards. Use greater tubercle of humerus and acromion of scapula as landmarks: Run the finger down the scapula of the animal. The first protuberance felt is acromion; the second protuberance felt is greater tubercle. Insert the needle just cranial and distal to the acromion and direct the needle distally to enter the shoulder joint.
4. Tarsal joints

Tarsal effusions are easiest to locate when running the fingers along the Achilles tendon from proximal to distal. To tap the tarsal joint, partially flex the tarsus. Palpate the lateral malleolus of fibula and Achilles tendon. Insert the needle plantar from malleolus and cranial to the Achilles tendon in a craniodistal direction to enter the joint.

5. Lateral Stifle Joint

A helper who partially flexes and rotates the leg laterally may be necessary to tap the stifle joint. Palpate the patella, patella ligament and tibial tuberosity as landmarks for this procedure. Insert the needle lateral to patella tendon, distal to the patella and proximal to the tibial tuberosity. Direct the needle proximal and medial. It is helpful to apply digital pressure on the medial site of the stifle joint to push any effusion to the lateral side. The stifle joint can also be tapped on the medial side. For this procedure the needle is inserted medial to the patella ligament and directed proximal and lateral.

6. Hip Joint

The coxofemoral joint is hard to palpate and tap as there are several muscles overlaying this joint. Care should be taken not to injure the sciatic nerve, which passes from the medial to the lateral ischium at the greater ischiatic notch and runs medial to the greater trochanter and caudal to the femur distally. The patient should be positioned in lateral recumbency with the pelvis parallel to table. The hip should be slightly abducted and the femur rotated medially. The greater trochanter of the femur serves as landmark. The needle is inserted dorsal to greater trochanter and directed medioventrally towards the femoral head. During the procedure, the needle may contact the acetabular rim or the femoral neck. If this happens, the needle should be redirected.

What to do after joint fluid is obtained

Joint fluid is produced by the synovial membrane lining the joint capsule. Disruption of this production may change the character of the fluid. A macroscopic examination of the joint fluid may already establish whether joint disease is present or not. Physiological joint fluid:

In a normal patient, the fluid is clear, colourless to slightly yellow and viscous. As mentioned previously, an amount of 0.01-1 ml may be obtained from a normal joint, depending on the patient’s size and the joint aspirated. Normal joint fluid should form a ropy strand of 2.5-5 cm when drawn from a glass slide. This should be tested (“string test”). If blood contamination is present, the blood will not mix with the joint fluid, but rather form streaks. The protein content of joint fluid should be less than 3 g/dL and the pH varies between 7 and 7.8. The total nucleated cell count within joint fluid should be less than 3000 cells/mm³. Polymorphonuclear cells should represent less than 10% of the total cell population.

Changes in colour, volume, viscosity, cell count or biochemical parameters may represent pathological conditions, such as septic arthritis, immune-mediated arthropathies, neoplasia or others.
To establish whether septic arthritis is present, joint fluid should be cultured (submit in plain vials, on culture swabs or blood culture bottles). The use of blood culture media is recommended particularly in cases where slowly growing organisms are expected or empirical antibiotic therapy was performed prior to the joint tap. A negative blood culture does not completely rule out a septic arthritis. To use blood culture bottles proceed as follows:

To avoid growth of contaminants, joint fluid needs to be introduced in a sterile manner as follows: After obtaining joint fluid, change the needle on the syringe to a new sterile needle. Clean the top of the blood culture bottle with plenty alcohol with or without sterile swabs. Introduce the needle into the rubber top of the blood culture bottle. There is usually a negative pressure in the blood culture bottle that will draw the sample into the liquid. Store the bottle away from sunlight at room temperature or in the fridge and ship to the lab as soon as possible.

PART II: Bone marrow aspiration

When is a bone marrow aspirate indicated?

The bone marrow aspiration is an ancillary procedure to diagnose and distinguish different bone marrow diseases, that is, hyperplasia, hypoplasia, aplasia, dysplasia and/or neoplasia of red and/or white blood cell lines. It is indicated when a definitive diagnosis and therapy cannot be obtained by other diagnostic procedures and when these procedures indicate bone marrow disease. This is the case if one of the following is present:

• Repeated complete blood counts including blood smears and other haematological tests show persistent abnormalities.
• Severe persistent unexplained nonregenerative anaemia
• Persistent neutropenia
• Nonregenerative thrombocytopenia
• Pancytopenia
• Atypical cells in blood
• Hypercalcaemia without identifiable cause from other diagnostic tests (last resort test)
• Hyperproteinaemia/ monoclonal gammopathy

A bone marrow aspirate may also be performed to stage lymphosarcoma.

A bone marrow aspirate is contraindicated when at least one of the following is present:

• Regenerative anaemia
• Regenerative thrombocytopenia
• Cytopenias caused by concurrent or recent drug administration (e.g. quinidine, sulfadiazine, cephalosporins, chemotherapeutics)

Please note, a regenerative response of the bone marrow to the loss of blood cells (e.g. of erythrocytes in immune-mediated anaemia, extravasation) is only seen after 2 to 5 days. A bone marrow aspiration should only be performed after this refractory period (i.e. not in acute and peracute cases).
What is needed to perform a bone marrow aspiration?

To perform a bone marrow aspiration, following material is necessary:

- Material for surgical site preparation (clippers, swabs, povidone-iodine or chlorhexidine solution)
- Local anaesthetic (e.g. lidocaine)
- Sterile gloves
- Bone marrow needle (see below)
- Scalpel blade (no. 11 or 15)
- EDTA tubes
- Sterile saline
- Syringes (10 ml)
- Pre-cleaned slides
- Nonabsorbable suture material

Illinois sternal needle, Jamshidi, Rosenthal, Cook intraosseus infusion needles or 18 gauge needles may be used to obtain bone marrow aspirates. Cook needles can be used to give intraosseus infusions. The Stylet of Cook needles is shaped like a corkscrew-handle, which is very comfortable during use. Cook infusion needles and Illinois sternal needles are quite short, which is disadvantageous in large or obese dogs. Jamshidi needles may also be used for bone marrow core biopsies (histopathological sample). A 18 gauge hypodermic needle can be used in cats with thin bones. The disadvantage of using needles is that the inner needle lumen may block with cortical bone (thus no sample can be aspirated). To avoid blockage of any bone marrow needle, make sure that the Stylet fills the lumen of the needle completely.

The use of a 10 ml syringe allows a greater negative pressure during aspiration. Shortly before the bone marrow aspiration is performed, EDTA coated syringes should be prepared. This is done by filling a 5 ml EDTA tube with saline. The saline is aspirated into the syringe and emptied into a second 5 ml EDTA vial and then aspirated again into the syringe. Finally, the syringe is emptied.

The pre-cleaned slides can be leaned at an angle of approximately 60º to allow a bone marrow drop placed on the slide to run down the slide with gravity (Figure 1). Alternatively, smears may be performed once the bone marrow drop is placed on the slide.

Figure 1 Slides are placed on an angle, to allow the bone marrow to run downwards with gravity
Preparation on the day and performing the bone marrow aspirate

Results of a complete blood count including blood smear, taken within the last day, should be interpreted and should show persistence of the noted abnormality immediately before the bone marrow aspirate is performed. In addition, some laboratories prefer to have an EDTA blood sample supplied at the time of the bone marrow sample is submitted. This is to allow concurrent interpretation of smears. The animal should be appropriately restrained. This may be achieved with a sedative or short general anaesthetic. Most patients that undergo this procedure are critically ill, requiring an appropriate anaesthetic protocol and monitoring. In quiet/very weak patients, bone marrow aspiration may be performed with local anaesthesia only.

A bone marrow aspiration may be performed on either the crest or wing of the ilium, the trochanteric fossa of the femur or the humerus. The iliac crest and trochanteric fossa may not be accessible in obese animals. In these patients, the humerus is the preferred site of collection. Lameness may result when bone marrow is sampled from the humerus or trochanteric fossa – particularly in small breed dogs. The lameness may persist for months to years.

The animal should be placed in the appropriate position (see below). If the dog is placed on a table, the table should be at a height that allows comfortable access to the site, having in mind that some downward pressure and rotating motion will need to be applied to penetrate the cortical bone. The chosen site should be prepared aseptically.

After preparation, the skin, subcutaneous layer, muscle and periosteum at the chosen entry site should be infiltrated with local anaesthetic (e.g. lidocaine up to a maximum dose of 4 mg/kg). A small incision is made into the skin at the entry site and the subcutaneous and muscular layers at the respective site are penetrated with the bone marrow needle. Once the needle reaches the periosteum, it is advanced through the cortical bone in a rotating motion. The needle is placed well when the bone (and animal) moves with the needle. When this is the case, the stylet should be removed and a (EDTA coated) syringe attached. Negative pressure is applied suddenly and released slowly until enough sample is in the syringe (0.5-2 ml is adequate). Avoid excessive repeats to minimise contamination with blood. The syringe is detached and the stylet is replaced into the needle. The needle is left in place until the quality of the sample has been examined (to allow taking further samples if the quality is not adequate). Drops of bone marrow are applied to several pre-cleaned slides (see above). Smears are prepared or the drop is allowed to spread with gravity (see above). Bone marrow clots very quickly and therefore preparation for cytological examination must be immediate after samples have been obtained. The remaining sample is stored in an EDTA vial.

A good bone marrow sample contains blood, fat droplets and white specks of bone marrow tissue. Slides should be assessed microscopically for good cellularity. They should be read within 24 hours or fixed with 5% methanol for few seconds. Do not expose slides to formalin or formalin fumes as this markedly decreases staining quality and makes interpretation difficult.

Performing a Bone Marrow Core Biopsy

The bone marrow core biopsy allows obtaining samples for both histopathology and cytology (via impression smear). The advantage of a histopathological sample is that stromal alterations and changes in cellularity can be evaluated more accurately. A
bone marrow core biopsy can be obtained with a Jamshidi needle. In addition to above mentioned material, a probe - for retrieving the sample from the needle - and a container with 10% neutral buffered formalin are needed. The preferred site for obtaining a bone marrow core biopsy is the iliac wing or iliac crest (see below).

To obtain the biopsy, prepare the animal and start the procedure as mentioned previously. When the needle reaches bone, withdraw the stylet and advance the needle further in a rotating motion. Aim to obtain a 2 cm biopsy specimen (this can be measured by carefully re-introducing the stylet into the needle). Stop when the needle emerges on the medial side of the iliac wing. When an appropriate sample is obtained, rotate the needle abruptly 360º to cut the biopsy specimen from the surrounding tissue. The needle can then be withdrawn in a rotating motion.

Recover bone marrow sample by pushing through proximal end of needle with the probe or stylet. If the biopsy is forced through the distal, more narrow, part of the Jamshidi needle, pressure artefacts may occur within the sample. Once the sample is obtained, impression smears may be performed. These should be kept away from formalin fumes (see above). The remaining sample is placed in 10% neutral buffered formalin.

Sites for Bone Marrow Aspiration, Core Biopsy and Intraosseus Infusion

1. **Iliac Crest or Wing**

   To perform a bone marrow aspirate of the iliac crest or iliac wing, the dog may be placed in lateral or dorsal recumbency. The iliac wing is palpated and the needle is introduced at the high point of the iliac crest (Figure 2). The needle is then directed ventrally and slightly caudally. The needle may slip over the periosteum on the lateral or medial side of the iliac wing. If this happens, the needle should be withdrawn to the high point of the iliac crest and the procedure should be restarted. Slipping occurs particularly often when the needle is blunt.

   The needle can also be directed into the dorsal 1/3 of the iliac wing (from lateral to medial). However, care must be taken not to penetrate the wing on the medial side.

Figure 2 Entry point and direction at iliac crest
2. Greater Trochanter of the Femur

The animal should be placed in lateral recumbency and the greater trochanter should be localised. The needle is introduced slightly medial to the greater trochanter and directed towards the stifle, parallel to the shaft of the femur (Figure 3). During the procedure, the femur is stabilised by holding the stifle with the second hand. This may be the preferred site for intraosseus infusion.

3. Proximal Humerus

The animal is positioned in lateral recumbency. The humerus is flexed and the elbow is rotated inwards. The needle is inserted little distal from the greater tubercle, which can be localised by running the finger down the scapula of the animal - the first protuberance felt will be the acromion, the second protuberance felt is greater tubercle. The needle is inserted at a 45° angle from craniodorsal to caudoventral.
When no Bone Marrow can be obtained

When no bone marrow can be obtained, the appropriate needle placement should be rechecked. The stylet may be replaced. The needle may be advanced or withdrawn a few millimeters and aspiration should be retried. If no sample can be obtained but positioning appears adequate, a different sample site should be tried. Alternatively, a small amount of sterile saline can be flushed through the needle and then re-aspirated. This may recover some cells.

It could also be that no bone marrow sample can be obtained, because the needle is blocked with cortical bone (particularly if no stylet is used or if the stylet is too small for the needle).

Finally, if no sample can be obtained from several sites despite adequate positioning, a pathological problem, such as myelophthisis, myelofibrosis or neoplasia may be present. A bone marrow core biopsy may help to evaluate whether this is the case.

Further reading