Collecting and Analyzing Cerebrospinal Fluid

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Indications

The principal reason for obtaining CSF is to diagnose inflammatory diseases involving the nervous system. Changes induced in CSF by non-inflammatory disease are nonspecific, but may contribute to a tentative diagnosis when other clinical findings are considered.

Contraindications

Should intracranial pressure be increased, removal of CSF may precipitate brain herniation. Neurologic clinical signs suggestive of increased intracranial pressure include stupor or coma and head pressing.

Technique

General anesthesia is required for cerebellomedullary cistern or lumbar cistern CSF collection.

Cerebellomedullary cistern

The animal may be positioned in either sternal or lateral recumbency. Landmarks are more readily identified when the animal is in sternal recumbency. However, lateral positioning may be necessary to obtain adequate CSF flow in some small dogs and cats and is also necessary if pressure is to be measured. Lateral positioning is preferred for these reasons.

The animal should be positioned on its right side (if the veterinarian is right-handed) with the head and neck flexed so that the head is perpendicular to the line of the neck. The head and neck should be parallel to the table top. Proper positioning is critical to ensure that the puncture is made on the midline. The cerebellomedullary cistern will be missed if the puncture deviates from the midline. The site of the puncture should be surgically prepared and draped to allow sterile palpation of all landmarks.

Landmarks for cerebellomedullary cistern puncture include the external occipital protuberance (cranial and midline marker) and the widest point of the transverse processes of the atlas (caudal marker). The puncture is made on the midline, midway between the external occipital protuberance and the widest point of the transverse processes of the atlas. A 22 gauge 1 1/2" spinal needle is adequate for most dogs and cats. As the needle passes through each tissue plane, the stylet should be removed to see if CSF is present. This reduces the possibility of inadvertent penetration of the spinal cord. Slight resistance is usually met when the needle contacts the occipital-atlanta membrane just prior to penetrating the dura mater. If bone is encountered, the needle should be withdrawn slightly and redirected either cranially or caudally. When improperly positioned, the needle has usually been placed too far cranially.

When pressure is not measured, CSF should be collected directly from the needle when the puncture is achieved. Attachment of a syringe to the needle for aspiration should be avoided as excessive suction can cause hemorrhage. Aspiration of fluid as it drips from the needle is preferred. Although some recommend use of a glass syringe, sterile disposable plastic syringes can be used. One milliliter of CSF is adequate for cytology and protein quantitation. Another milliliter of CSF should be collected in a sterile syringe, capped, and submitted for culture if results of cytological examination indicate possible infection.

Bloody CSF often will clear if allowed to drip. If the CSF fails to clear, evaluation may still be rewarding. If pure blood is obtained, the needle should be discarded and the procedure repeated. The animal's position should be reevaluated and landmarks should be palpated again. When blood is obtained more than twice, the procedure should be stopped and repeated several days later.

Lumbar cistern

Lumbar puncture is preferred for myelography and when a solitary thoracolumbar spinal cord lesion is suspected. Both the ventral and dorsal subarachnoid spaces in the caudal lumbar area have been recommended for collection of CSF and myelography. Use of the ventral subarachnoid space involves passing the spinal needle through the cauda equina or caudal lumbar spinal cord. This is associated with an area of myelomalacia that corresponds to the path of the needle but rarely causes detectable neurologic dysfunction. The risk of inadvertent movement of the needle and subsequent injection of contrast medium into the spinal cord at the time of myelography is greater dorsally than ventrally where the point of the needle is positioned against the floor of the spinal canal. For this reason, the ventral subarachnoid space generally is used for CSF collection and myelography.

The animal should be positioned on its right side (if the veterinarian is right-handed) with the caudal lumbar area mildly flexed. The thoracolumbar vertebral column should be parallel to the tabletop. The puncture should be attempted first at L6-L7 in dogs and L7-S1 in cats. If CSF cannot be obtained at either of these sites, L5-L6 should be used.

Landmarks for lumbar puncture include the dorsal spine of L7 which can be palpated between the tuber coxae of the pelvis and the small dorsal spines of the sacrum which can be palpated caudal to the dorsal spine of L7. When L6-L7 is the puncture site, the needle (22 gauge 2 1/2 or 3 1/2 inch needle) is inserted lateral to the dorsal spine of L7 and directed in a slight cranioventral dirction (20 degrees from vertical) to the lumbar spine. When bone is encountered, the needle should be moved either cranially or caudally until the interarcuate depression is located. The needle is then forced through the interarcuate space into the dorsal subarachnoid space or through the spinal cord or cauda equina so that the point rests on the floor of the vertebral canal. If CSF is not obtained from the

ventral subarachnoid space, the needle should be withdrawn slightly until the ventral subarachnoid space is entered. In larger dogs, the dorsal lamina of L6 may overlie the interarcuate space, preventing insertion of the needle if this technique is used. In these dogs, the needle should be inserted caudal to the dorsal spine of L7 and directed cranially so that it passes ventral to the dorsal lamina of L6. Both techniques can be used at L5-L6 and L7-S1.

Evaluation

Gross examination

Normal CSF should be clear and colorless. Turbidity is usually due to large numbers of cells or bacteria. Clotting may occur if fibrinogen is present. A grossly bloody sample is due to a "traumatic tap" or subarachnoid hemorrhage. Crenated erythrocytes occur in both situations and are of no significance. In a traumatic tap, the amount of blood will vary, being greatest initially and least at the conclusion of the sampling. Xanthochromia, a yellowish color in the supernatant of centrifuged CSF, usually suggests prior subarachnoid or intracerebral hemorrhage. Gross blood due to subarachnoid hemorrhage may resolve within 24 hours but generally persists for 7 to 14 days.

Cell count and differential

This must be done within 30 minutes after sampling, as cells autolyze rapidly. Techniques described below can be done in your own hospital with minimal equipment. Alternatively, CSF samples can be submitted to an outside laboratory; however, keep in mind that the cell count and differential must be done expeditiously.

On one side of the counting chamber of a hemocytometer, directly plate CSF. If, as is normally the case, there are only a few cells and RBCs and WBCs can be differentiated, cells on all nine squares should be counted. The cell count per ul is calculated by multiplying the number of cells counted by 1.1 (9 mm^2 squares X 1.1 to get to mm^3).

White blood cells are more refractive with reduced light and have a yellowish tint. They are also usually larger than RBCs. This may be difficult to discern in the case of lymphocytes. Macrophages will be very large, and thus easier to identify as nucleated cells.

If WBCs and RBCs cannot be differentiated, count all cells on direct plating, multiply by 1.1 to give the number of cells per ul. Then subtract the value for WBCs obtained below and derive the total RBC count.

1. Using a WBC pipette, draw CSF diluting fluid (10 ml of glacial acetic acid, 90 ml of distilled water, and 0.1 gm of crystal violet; CSF Diluting Fluid, Harleco, Gibbstown, NJ) to the 1.0 mark.
2. Fill with spinal fluid to the 11 mark.
3. Shake at least 2 minutes - discard 3 to 4 drops.
4. Fill both sides of the hemacytometer chamber and count all 18 squares under low power.
5. All RBCs should have been lysed by the diluting fluid. The cells counted are WBCs. Their cell type should be identifiable.
6. Total the WBCs counted in the 18 squares and multiply by 0.6 to get the count per ul.

Several techniques can be used to obtain a differential cell count. Ideally, samples are prepared using a cytocentrifuge. However, this instrument is expensive. Addition of a few drops of canine serum to the sediment prior to preparing the smear improves its quality. Alternatively, various sedimentation systems have been used. One employs a portion of a glass syringe affixed to a slide with paraffin. Cerebrospinal fluid is placed in the syringe and allowed to settle for 25 minutes, at which time the glass syringe is broken away. The sedimented cells are fixed with a either a spray (Profixx, Scientific Products, McGraw Park, IL) or immersed in methanol for 2 minutes. The slide is then stained and a differential white count is done.

Protein

Several methods may be used in commercial laboratories for determination of protein. A relatively simple test that can be used in your own hospital is termed the Pandy test. Briefly, it involves adding a ml of Pandy reagent (10 grams of carbolic acid crystals in 100 ml distilled water) to a test tube. Then add several drops of CSF and mix well. The resulting turbidity (subjectively graded as 1 - 4+) indicates the degree of globulin present.

Reference

W. Vernau, K Vernau, C Bailey: Cerebrospinal fluid. In Clinical Biochemistry of Domestic Animals, Sixth ed., JJ Kaneko, JW Harvey, ML Brus (eds). Elsevier, Boston. Pages 769-820.