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# Diagnostic Value of Cerebrospinal Fluid Evaluation in Veterinary Practice: An Overview

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**Abstract**

Collection and evaluation of cerebrospinal fluid is one of the most important aspects in investigation and diagnosis of various diseases with involvement of central nervous system and spinal cord. In veterinary practice, scanty information is available regarding biochemical and physiological laboratory tests and biochemical referral values of biological ingredients of cerebrospinal fluid, hence this paper may be informative for researchers and veterinary practitioners.

*Keywords:* Cerebrospinal fluid; atlanto-occipital; lumbosacral

## Introduction

Cerebrospinal fluid (CSF) is the main component of the brain extracellular space and participates in the exchange of many biochemical products in the central nervous system (CNS). Consequently, CSF contains a dynamic and complex mixture of proteins, which reflect physiological or pathological state of CNS. Changes in CSF protein reflect pathological changes in the brain and thus contribute to a better understanding of the patho-physiology of the underlying neurological disorders (Gawinecka *et al*., 2010). Although a definitive diagnosis of CNS disease based only on CSF analysis is rarely possible, CSF evaluation can be helpful in differentiating traumatic from infectious spinal lesions and toxic or metabolic lesions from bacterial meningitis (Scott, 1992). It has been suggested that CSF examination may be useful for ancillary diagnosis of a spinal fracture (George, 1996). Regardless of the cause, early identification and treatment of CNS disease are essential, and CSF analysis can be helpful for an accurate diagnosis of CNS diseases. Scanty information is available regarding biochemical and physiological laboratory tests and biochemical referral values of biological ingredients of CSF hence, this paper may be informative for researchers and veterinary practitioners.

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## Collection of CSF

For collection of CSF the materials required are 12.5 cm long 14 G needle with a stylet, 3 inch 16 G spinal needle, cotton swab, BP handle with knife, rectified spirit, sterilized test tubes, sharp razor, surgical gloves etc in sterilized condition and aseptic precautions should be taken during collection.

## Methods of collection

The CSF is collected from either of the two sites, i.e. Cisterna magna or atlanto-occipital puncture and Sub lumbar or lumbosacral puncture.

*a) Atlanto-occipital puncture*

The CSF can be obtained by atlanto-occipital puncture in lateral recumbency during general anaesthesia in dogs, cats and equine (Ettinger and Feldman, 2005; Schwarz and Piercy, 2006), and done in standing position or in lateral recumbency under sedation (xylazine hydrochloride at 0.05 mg/ kg, IV) and local analgesia (2% lignocaine hydrochlo-

ride) in ruminants with following steps:

- Restrain the animal.
- The head is flexed at the atlanto-occipital joint approximately 90º (ensure that airway remain patent during flexion), so that the long axis of the head is perpendicular to spine.
- Clip, clean, and sterilize the selected area.
- Puncture in the midline along an imaginary line drawn between the cranial borders of the atlas wings.
- Use a 3-4 inch long and 16 G spinal needle with a stylet and insert it slowly at the cranial edge of the wings of atlas. Direction of the needle should be parallel to the long axis of the head. When the needle enters subarachnoidal space, loss in resistance is felt.
- As the needle enters into atlanto-occipital joint, remove the stylet so that the CSF flows out and collect approximately 1 to 2 ml of CSF.

*b) Lumbosacral puncture:*

The collection is done under local analgesia (2% lignocaine hydrochloride) in the standing position.

- Presurgical and aseptic precautions are taken and the depression between the dorsal process of last lumbar vertebra and cephalic end of median crest of sacrum is palpated.
- Needle is passed at this site. Insert the needle vertically, then slightly oblique by applying gradual pressure in forward and backward directions. As the needle enters in subarachnoid space comparatively less resistance is felt.
- Animal must be tied firmly to avoid damage to the spinal cord. CSF collection is done by removing the stylet, apply a syringe and suck the fluid.

**Examination of CSF**

The CSF is examined for following tests

*a) Physical examination (Table 1)*

Colour:

Normal CSF is clear, colourless, free of flocculent material, and with the same viscosity as water (Smith and George, 2002; Ameri and Mousavian, 2007). Any change in colour generally represents an abnormality. More than 600 RBC/µl gives CSF a red discolouration. If the haemorrhage is recent or iatrogenic, CSF usually becomes colourless with centrifugation (Hayes, 1987). However if it persists, earlier haemorrhages should be suspected.

Turbidity:

Bacteria, fungi or epidural fat may occasionally cause it to appear turbid in the absence of pleocytosis (Lawrence, 2005). Usually through, slight turbidity is appreciated with as few as 200 WBC/µl or 400 RBC/µl (Hayes, 1987). Viral encephalitis, trauma, tumors, or abscesses may produce turbidity in CSF due to large amounts of protein, fibrin, or cells.

Coagulation:

Normal CSF does not coagulate. CSF may clot if it contains increased fibrinogen (occasionally seen in suppurative meningitis or if the sample is significantly contaminated with blood (Lorenz and Kornegay, 2004).

Table 1. Physical examination of CSF

| Parameters | Observation | Inference |
|---|---|---|
| Colour | Clear, colourless, watery and free of flocculent material | Normal |
| | Red | Puncture of blood vessel during collection |
| | Dull red / brownish | Intracranial haemorrhage, cranium fracture |
| | Yellow (xanthochromic) | Presence of bile pigments (jaundice), old haemorrhage in CNS |
| | Greyish or greenish | Due to infection leading to pus formation |
| Turbidity | Clear, transparent | Normal |
| | Hazy, ground glass like | Presence of cells/ white clots appearance (pleocytosis) |
| | Cloudy/purulent | Encephalitis, bacterial meningitis. |
| | Red turbid | Puncture of blood vessel during collection |
| Coagulation | No coagulation | Normal |
| | Coagulation | Presence of abnormal amount of proteins especially fibrinogen in cases of suppurative meningitis |
| | Blood (in large quantities) | Internal haemorrhage or improper collection. |

*b) Chemical examination (Table 2)*

Protein:

CSF protein concentration is one of the most sensitive indicators of a pathological process within the CNS. In the case of bacterial infection of the CNS, combined interpretation of the protein concentration and the differential WBC count could be useful to identify the disease (Scott, 1992). In normal CSF, protein concentrations are very low and consist almost entirely of albumin. The protein concentration have been reported in CSF from sheep (8–70 mg/dl; George, 1996), cattle (23.4–66.3 mg/dl; Welles *et al*., 1992), and dog (11-55 mg/dl; Hoerlin, 1978). In neonatal foals, a more permeable blood-CSF barrier is believed to account for their higher CSF protein concentration, reflecting both increased IgG and albumin (Andrews *et al*., 1994). Ponies are also reported to have higher CSF protein concentrations than horses (Mayhew *et al*., 1977). Increased protein concentrations may occur with damage to the blood brain barrier or increased local production of immunoglobulins.

Glucose:

The quantitative estimation of CSF glucose is done by the Folin–Wu technique. Glucose concentration in CSF depends upon the blood concentration, the rate of glucose transport into the CSF, and the metabolic rate of the central nervous system (Bailey and Vernau, 1997). Therefore, glucose in CSF should be compared with simultaneously measured serum glucose. In healthy animals, glucose concentration in CSF is about 80% of that in the serum (George, 1996; Bailey and Vernau, 1997). The glucose concentration of CSF has been reported in sheep (48–109 mg/dl), cattle (20–40 mg/dl), goats (24–40 mg/dl) (George, 1996), and dogs (61-116 mg/dl; Hoerlin, 1978). Glucose concentrations of CSF are somewhat higher in foals, but decreases rapidly with age (Furr and Vender, 1994).

An increased glucose concentration in the CSF (hyperglycorrhacia) is seen in association with any disease leading to hyperglycemia (Diabetes mellitus), encephalitis, spinal cord compression, brain tumors or brain abscess (Ettinger and Feldman, 2005). A decreased CSF glucose concentration (hypoglycorrhacia) in animals is associated with systemic hypoglycemia or bacterial meningitis and marked neutrophilic pleocytosis. Consumption of glucose by bacteria and neutrophils is a likely mechanism (George, 1996; Bailey and Vernau, 1997). Dogs with nervous distemper have a low CSF glucose level (Ettinger and Feldman, 2005).

Chloride:

The levels are normally higher in CSF than in blood. Normal CSF values in domestic animals ranges between 650-850 mg/dl. Lower values are seen in pyogenic meningitis, protracted vomiting, advanced pneumonia, hypochloremia, while normally higher values of chloride in CSF are recorded than in serum.

Sodium:

The concentration of sodium in CSF is similar to the value in serum. CSF concentration of sodium >160 mEq/L is considered diagnostic for salt poisoning (Jamison and Lumsden, 1988).

Cholesterol:

Haemorrhages in the CNS, tumors, meningitis and brain abscess lead to an increase in cholesterol content. Usually normal cholesterol level is very low and values recorded in Horse: 0.36 - 0.55 mg/dl, and Goat: 0.51 mg/dl.

Enzymes activity:

Increased levels of CSF ALT (alanine aminotransferase): 20.1(9-46 unit) and AST (aspartate aminotransferase): 13.7 (2-32 units) have been observed in dogs suffering from distemper with involvement of CNS, purulent meningitis, and cerebral infarction. Lactic dehydrogenase enzyme level of CSF is also increased in bacterial meningitis, metastatic carcinoma, lymphoid tumor, subarachnoid haemorrhage and cerebral infarction. A marked elevation in the CPK (creatinine phosphokinase) is also seen in certain neurological conditions.

Calcium:

Normally the calcium is lower in CSF than in serum. Increased level of protein bound calcium in CSF indicates disturbance in blood brain barrier.

Table 2. Chemical examination of CSF

| Tests | Observation | Inference |
|---|---|---|
| Foam test<br>Take CSF in test tube and shake the test tube at least for 5 minutes | Slight foam that disappears after few minutes<br>More foam that remains<br>Protein levels Increased | Normal Protein levels |
| Sulfosalicylic acid test (SSA)<br>3 ml of 3% SSA + 1 ml CSF<br>Mix and allow to Stand | Increase in the turbidity | Presence of proteins |
| Nonne - Apelt test<br>1 ml saturated ammonia solution + 1 ml CSF<br>Do not mix. Allow to stand | White to greyish ring at the junction of two fluids | Presence of increased amounts of globulin in CSF which is seen in<br>1. Encephalitis,<br>2. Meningitis,<br>3. Neoplasia,<br>4. Haemorrhage,<br>5. Hydrocephalus,<br>6. Tissue destruction,<br>7. Uraemia,<br>8. Toxoplasmosis,<br>9. Pneumonia |
| Pandy's test<br>1 ml saturated phenol or Pandy's reagent, 1-2 drops of CSF Shake (Pandy's reagent is prepared by dissolving 10 grams of pure phenol in 150 ml of distilled water) | White cloudy or turbid | |

*c) Cytological examination*

Normal CSF consists almost entirely of mononuclear cells in which small lymphocytes are the predominant cell type (Bailey and Vernau, 1997).The total cell counts of the CSF must be estimated within 20 minutes of collection, since the cells degenerate rapidly. The estimation of the number of cells is done as for the determination of WBC's of the blood. The total numbers of cells which are obtained are then multiplied by 0.6 to get number of cells in one cu mm of the CSF.

Normal count

| Species | Counts (Cells/cu mm) |
|---|---|
| Cattle, sheep and pig | 0 - 15 |
| Dog | upto 25 |
| Horse | upto 23 |

Pleocytosis or increased number of WBC's are seen in inflammatory conditions of brain, spinal cord or meninges, abscess of brain or spinal cord, encephalitis, chronic inflammatory conditions, toxic or degenerative conditions.

Differential Count of CSF can be done by preparing the smear from CSF. Smear is stained with leishmans stain after drying and examined under microscope. Neutrophilia indicates pyogenic or bacterial infection, abscesses in brain, bacterial meningitis, encephalitis and haemorrhage, while lymphocytosis is observed in uremia, toxemia, chronic viral and fungal infection (Table 3).

Table 3. Anticipated cerebrospinal fluid analysis results by disease status.

| Disease | Protein mg/dl | Cells /µl | Cells-type |
|---|---|---|---|
| Normal | <40 | <10 | Lymphocytes |
| Viral | 40–100 | 50–200 | Monocytes lymphocytes |
| Bacterial | >100 | >200 | Neutrophils |
| Degenerative | <40 | <10 | Monocytes |

*d) Bacteriological examination*

It is carried out when the CSF cell count and protein contents are high. The organisms are isolated in CSF and identified by cultural methods.

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