

Techniques of collection, analysis and interpretation of cerebrospinal fluid in dogs and cats: Literature review**Técnicas de colheita, análise e interpretação de líquido cefalorraquidiano em cães e gatos: Revisão de literatura**

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ABSTRACT

Analysis of the cerebrospinal fluid is useful in the evaluation of the brain and spinal cord and can help in the diagnosis of some neurological disorders, being an important tool in the investigation of these diseases. Because it is a procedure that presents a certain risk to the patient, it is necessary to have adequate knowledge of the anatomy and physiology of the region, of the indications and contraindications of the examination and of the technique to perform it. The laboratory analysis of the sample should be as complete as possible, always being performed by a qualified professional, emphasizing the physical examination, total and differential cell counts and glucose and protein analyses, and these findings should be correlated with the clinical examination of the patient, thus helping in the elaboration of a more accurate diagnosis.

Keywords: veterinary clinical pathology, neurology, dog, cat, liquor**RESUMO**

A Análise do líquido cefalorraquidiano é útil na avaliação do encéfalo, da medula espinhal e pode auxiliar no diagnóstico de alguns distúrbios neurológicos, sendo uma importante ferramenta na investigação destas enfermidades. Por ser um procedimento que apresenta certo risco para o paciente, deve-se ter conhecimento adequado da anatomia e fisiologia da região, das indicações e contraindicações do exame e da técnica de realização do mesmo. A análise laboratorial da amostra deve ser a mais completa possível, sendo sempre realizada por profissional capacitado, dando ênfase ao exame físico, contagens total e diferencial de células e análises de glicose e proteínas, devendo estes achados serem correlacionados com o exame clínico do paciente, auxiliando assim na elaboração de um diagnóstico mais preciso.

Palavras-chave: patologia clínica veterinária, neurologia, cão, gato, liquor

1 INTRODUCTION

The cerebrospinal fluid (CSF) or liquor is a clear fluid present in the cerebral ventricles, the central spinal canal and the subarachnoid space, whose function is to nourish and protect the Central Nervous System (CNS). In its composition, there are almost no blood cells and little protein. Its speed of formation, flow and absorption is high enough to promote its replacement several times a day (CUNNINGHAM, 1997; PLATT & GAROSI, 2012; POLIDORO et al., 2018). It is produced primarily by the vascular choroid plexus of the ventricular system and secondarily by ependymal cells and blood vessels of pia-arachnoid membranes. (COLES, 1984; CUNNINGHAM, 1997). The formation of CSF by the choroid plexus involves blood filtration through fenestrated capillaries in their interstitial space and active secretion of this filtered through the epithelium of the choroid plexus. (COLES, 1984; BRAUND, 1994; LAHUNTA & GLASS, 2009; POLIDORO et al., 2018). The amount produced depends on the species, age and size of the patient, and production of approximately 0.05cm³ and 0.02cm³ per minute was indicated for dogs and cats, respectively (BRAUND, 1994). The analysis of the CSF is an important complementary examination for the evaluation of patients with neurological problems (WHEELER & SHARP, 1999; PARRY, 2005).

Changes in the composition of the CSF are often observed in CNS diseases, especially if the area involved is in contact with the spaces where the CSF circulates, so the collection and analysis of the CSF are viable and effective means of access and evaluation of the nervous system, concerning the diagnosis and prognosis of its numerous diseases (BRYAN & BROBST, 1989; GAMA & SOBREIRA et al., 2005; POLIDORO et al., 2018).

If the appropriate technique is used, the procedure for obtaining the CSF is very simple and safe (TAYLOR, 2001). The fluid must be collected aseptically with the patient under anesthesia, by percutaneous insertion of a spinal or hypodermic needle until it reaches the subarachnoid space of the spinal cord. The collection can be performed in the cisterna magna (atlantoaxial space) or lumbosacral space (L6-L7 or L7-S1) (FENNER, 1998; MCDONNELL, 1998; WHEELER & SHARP, 1999; PARRY, 2005; SCOTT, 2005). Besides the ventricular formation of the CSF, there is the theory that there is a considerable production of CSF in the subarachnoid space. This space is called cisterna magna, being one of the places of CSF puncture in dogs and cats (COLES, 1984; MOURA et al., 2004). Functionally, the CSF protects the brain and spinal cord from trauma, modulates variations in intracranial pressure, and assists in maintaining ion concentrations at appropriate levels (COLES, 1984). Brain and CSF densities are similar. Because of this, the brain floats in the fluid. Since the

CSF is in balance with the extracellular fluid in the brain, it also helps to maintain a constant extracellular environment for the neurons and glial cells in the brain. The CSF can also serve as a conduit for some brain polypeptide hormones and other substances and has a defense function against infectious agents since it distributes the defense cells homogeneously and also facilitates the rapid spread of immunoglobulins. Besides the function of protection of the CNS, the CSF acts as a source of nutrients and removes metabolites from the CNS (BRYAN & BROBST, 1989; CUNNINGHAM, 1997; MOURA et al., 2004; GAMA & SOBREIRA et al., 2005).

Although CSF analysis in general is useful to determine the presence of diseases of the nervous system, by itself it does not lead to a specific etiological diagnosis (BAGLEY & MAYHEW, 2002).

This work has as objective to make a literature review about the techniques used for the CSF collection in the small animal clinic, the sample analysis, and the interpretation of the results obtained in this analysis, alerting the veterinarian about its main aspects.

Indications and contraindications of the examination

Initially, the collection and laboratory examination of the CSF is indicated whenever there are disorders in the CNS (COLES, 1984; BRYAN & BROBST, 1989; SARMENTO & TUDURY et al., 2000). Certain conditions of the CNS, particularly infectious and inflammatory brain diseases, can alter the CSF and its laboratory analysis of various changes in nerve roots, spinal cord, brain stem, and brain can be of great help. Inflammation, neoplasia, and compression of the spinal cord often show the greatest changes in the CSF (CHRISMAN, 1985; MCDONNELL, 1998; DEWEY, 2003).

Occasionally, the examination may be useful as a prognostic method for the evaluation of the disease and for monitoring the response to treatment. Also, removal of the CSF may be a therapeutic indication for the slowing down of clinical signs associated with CNS disorders such as hydrocephalus (COLES, 1984). CSF examination is also indicated in dogs and cats with suspected intracranial disorders as a cause of seizures, with fever and paravertebral pain, or with progressive signs of mental deterioration (FENNER, 1998; TAYLOR, 2001), and should always be considered in patients with spinal disorders where radiography and myelography are normal, in patients with multifocal signs, and also in those with suspected polyneuropathy (WHEELER & SHARP, 1999; DEWEY, 2003).

CSF collection is not recommended after trauma because, besides already having a diagnosis, it can be dangerous. The collection is often dangerous under conditions that cause increased intracranial pressure (ICP), such as cerebral edema, hydrocephalus, or intracranial hemorrhages

because it may increase the risk of cerebral herniation (BROBST & BRYAN, 1989; RASKIN & MEYER, 2001; TAYLOR, 2001; MOURA et al., 2004). The CSF should not be punctured in patients with cerebral hernia. It can be recognized by a rapid deterioration in the state of consciousness, changes in pupil size from small and reactive to fixed and dilated, loss or decrease in the oculoccephalic reflex and pathological breathing (SCOTT, 1997; FENNER, 1998; MCDONNELL, 1998; WHEELER & SHARP, 1999).

The spinal puncture should not be performed if, for any reason, general anesthesia is contraindicated or in patients with severe coagulopathy in which bleeding complications are very likely (TAYLOR, 2001; RASKIN & MEYER, 2001). Anesthesia is a risk, especially in cases of dehydration, respiratory pairings, or when the respiratory center bulbar is accidentally injured during pressure (SARMENTO & TUDURY et al., 2000).

If there is skin infection at the puncture site, as well as the suspicion of viral diseases or bacteremia, the CSF collection is not recommended because of the risk of meninges contamination (COLES, 1984; SARMENTO & TUDURY et al., 2000). The puncture can injure vessels, leading infectious agents to encounter the nervous tissue (SARMENTO & TUDURY et al., 2000).

The patients who present fractures and dislocations involving the atlantooccipital joint or bones close to the trunk-brain and cerebellum will also have the CSF collection contraindicated, since the positioning for the collection may generate spinal compression and cardio-respiratory arrest (SARMENTO & TUDURY et al., 2000), due to the danger of positioning the patient (WHEELER & SHARP, 1999).

The CSF should also not be collected from patients who underwent myelography within forty-eight hours because, due to the inflammatory reaction caused in the subarachnoid space, the analysis of the CSF will be altered in this period, making sample interpretation difficult or impossible. (MCDONNELL, 1998).

Analysis of CSF after convulsions can produce interpretation errors, as they alter the blood-brain barrier, producing increases in proteins and total cells (SARMENTO & TUDURY et al., 2000).

CSF analysis is generally not recommended in patients with metabolic abnormalities, obvious disc diseases, CNS anomalies, or neurological signs caused by trauma (TAYLOR, 2001).

The main indications and contraindications for the CSF collection are listed in **Table 1**.

Table 1: Main indications and contraindications of CSF collection in dogs and cats

INDICATIONS	CONTRAINDICATIONS
Changes in nerve roots, brain stem, and brain	After traumas
CNS neoplasms	Conditions causing increased ICP (cerebral edema, hydrocephalus, intracranial hemorrhages)
Changes in the spinal medulla (compression)	General anesthesia contraindicated
Infectious brain diseases and inflammatory	Cerebral hernia
Suspect of intracranial disorders	Skin infection at the puncture site
Spinal disorders in which radiography and myelography are normal	Fractures and dislocations in the atlantooccipital articulation or bones near the brainstem or cerebellum
Multifocal signs	Doing myelography in less than 48 hours
Suspected polyneuropathy	Convulsions, metabolic abnormalities, obviously disk diseases and CNS anomalies

Source: COLES, 1984; SCOTT, 1997; FENNER, 1998; MCDONNELL, 1998; WHEELER & SHARP, 1999.

Initial sample collection procedures

To collect the CSF, the patient must first be under general anesthesia and properly intubated (BRAUND, 1994; FENNER, 1998; MCDONNELL, 1998; WHEELER & SHARP, 1999; SARMENTO & TUDURY et al., 2000; TAYLOR, 2001; PARRY, 2005; SCOTT, 2005; LUCAS & SACCO, 2008). The nuchal region, from the occipital protuberance to the second cervical vertebra (C2), should be prepared by trichotomy and antisepsis with bactericidal substances such as 70% alcohol and a chlorhexidine (TAYLOR, 2001). If a lumbar puncture is decided, this area should also be trichotomized and prepared aseptically (SCOTT, 1997; WHEELER & SHARP, 1999). Sterile needles are used for the collection, and the use of sterile surgical gloves is recommended. An assistant is needed to keep the patient in the proper position while the puncture is performed (WHEELER & SHARP, 1999).

Spinal needles are preferred for CSF collection, but hypodermic needles can be used (MCDONNELL, 1998; WHEELER & SHARP, 1999; LUCAS & SACCO, 2008). It is recommended to have four sterile test tubes for coagulation and/or plastic test tubes with ethylenediaminetetraacetic acid (EDTA) to collect and transport the sample. A clinical pathology laboratory that can analyze the sample within sixty minutes of collection should be available. If this is not possible, samples should be kept refrigerated at 5 to 12 celsius degrees (MCDONNELL, 1998; ALMEIDA et al., 2007).

Places to collect the sample: advantages x disadvantages

The two areas used for CSF collection are the cisterna magna and the spinal lumbar region (FENNER, 1998; WHEELER & SHARP, 1999; TAYLOR, 2001) in the space between L5 and L6 in dogs and L6 and L7 in cats (WHEELER & SHARP, 1999).

Obtaining the CSF of the cisterna magna is much easier, as it is more accessible and has a higher volume. In the lumbar region, the collection is more difficult due to the small subarachnoid space and the presence of the vertebral arches making blood contamination frequent (COLES, 1984; BROBST & BRYAN, 1989; BRAUND, 1994; WHEELER & SHARP, 1999), however, as the blood flow is in the cranial-caudal sense, the abnormal CSF is more likely to be present caudally to the lesion, thus, the CSF obtained in the lumbar region is diagnostically more useful (WHEELER & SHARP, 1999; BAGLEY & MAYHEW, 2002).

The main differences in the laboratory analysis of samples collected from each puncture area are described in Table 2, but the choice of puncture area depends on the clinical indication (FEITOSA et al., 1997).

Table 2: Differences in laboratory analysis of samples collected by cisternal and lumbar puncture from healthy dogs

	Cisternal Punction	Lumbar Punction
Aspect		
Color	No significant differences	No significant differences
PH Density		
Global RBC count		
Leukocyte count	Bigger	Smaller
Total Protein	Smaller	Bigger

Source: FEITOSA et al., 1997; Scott, 1997; BAILEY & HIGGINS, 1986; SARMENTO & TUDURY et al., 2000; TAYLOR, 2001

In cats it is recommended the cisternal puncture to remove the CSF, not more than 0.5 to 1.0mL of fluid, because this patient will be susceptible to meningeal hemorrhage. In kittens, not more than 10-20 drops of CSF should be removed (COLES, 1984; DEWEY, 2003).

CSF collection technique and its complications

To collect the CSF through the cisterna magna, the patient's head is positioned at a 90° angle to the spine (SCOTT, 1997; SARMENTO & TUDURY et al., 2000). For the right-handed clinician, the patient should be placed in the right lateral decubitus position; the nose should be raised slightly so that it is parallel to the table surface. This positioning will separate the occipital condyles from the atlas, thus increasing the area for puncture. The neck should be positioned on the edge of the table. The collector should be lowered so that the atlantooccipital area is at eye level (COLES, 1984;

MCDONNELL, 1998). With the thumb and third finger of the left hand, the cranial edges of the atlas wings should be palpated, and an imaginary line should be drawn by its most cranial face (WHEELER & SHARP, 1999; TAYLOR, 2001). The puncture area will be in the midline of the patient, halfway between the occipital protuberance and the line joining the wings of the atlas, the needle is inserted perpendicular to the skin, in the midline, in a controlled manner to prevent the needle from penetrating too much (MCDONNELL, 1998; WHEELER & SHARP, 1999). The cisterna magna is reached by inserting a spinal needle in the center of the triangle formed by the occipital protuberance and wings of the Atlas (CHRISMAN, 1985). The mean distance between the skin and the subarachnoid space in the cervical spine varies with the size of the patient (FENNER, 1998).

The needle with the mandrel is inserted until the dura mater is penetrated and the liquor appears in the needle cube; a slight crack or dry noise can be heard when the needle penetrates the subarachnoid space inside the cisterna magna (SCOTT, 1997; MCDONNELL, 1998; WHEELER & SHARP, 1999). A small resistance can be felt when the atlantooccipital membrane and dura mater are simultaneously penetrated (BRAUND, 1994; SCOTT, 2005). In small dogs and cats, this resistance is more difficult to be perceived (SCOTT, 2005). The mandrel is removed, and the exit of the CSF is observed in the needle cannon. The needle is stabilized with the left hand while the stylet is removed with the right hand to prevent iatrogenic lesions in the medulla or cerebral trunk. If CSF is not observed after the initial removal of the mandible, the mandrel is replaced, the needle is moved forward and the procedure for observing CSF flow is repeated (MCDONNELL, 1998; TAYLOR, 2001; SCOTT, 2005). If two attempts are made to collect the CSF without success, the operator must take into consideration a lumbar puncture to avoid the risk of damaging the cervical spine (SCOTT, 2005).

Side movement and needle rotation should be avoided as it can cause spinal cord injury (SCOTT, 1997; TAYLOR, 2001). Care should be taken not to go too far with the needle and damage the spinal cord (PARRY, 2005), and not to flex the patient's neck excessively, which may partially occlude the endotracheal tube, and increase intracranial pressure by reducing jugular venous flow (SCOTT, 2005).

For a lumbar puncture, the patient is positioned in sideways decubitus. The lumbosacral region is flexed while the pelvic limbs are pulled cranially (SCOTT, 1997; SCOTT, 2005). It should be observed if the lumbar spine is parallel to the table and if there is no rotation of the spine (SCOTT, 1997). For large patients, the puncture is usually performed in the space between L5 and L6 in small dogs and cats (SCOTT, 1997; DEWEY, 2003). The point of entry is palpated by the location of the dorsal thorny process of L6 located between the wings of the ileum. As an assistant flexes the patient's

spine, the needle is directed perpendicularly to the spine and slightly to the side of the thorny L6 process to penetrate the inter arched ligament between L5 and L6. After insertion into the skin, the needle contacts the bone; the needle is then directed cranially or caudally to locate the inter arched depression of L5 and L6 and this is forced through the inter arched ligament of L5 and L6 and into the dorsal subarachnoid space (MCDONNELL, 1998). Sometimes, at this point, a muscle contraction of the pelvic limbs and/or tail is noted (DEWEY, 2003). The mandrill should be removed intermittently to assess progression. The flow of the CSF is slower in this region and blood contamination is more likely (SCOTT, 1997; WHEELER & SHARP, 1999; SCOTT, 2005). Most of the time, this procedure results in the penetration of the spinal cord and stopping the needle in the ventral floor of the vertebral canal. In this case, the needle is removed slowly to enter the ventral subarachnoid space (SCOTT, 1997; MCDONNELL, 1998; SCOTT, 2005).

The CSF must be collected by free flow, without aspiration (PARRY 2005). Normally the CSF will drip slowly from the needle in both puncture techniques. The first drops should be discarded avoiding skin cells and contamination of the sample (COLES, 1984; CHRISMAN, 1985). CSF aspiration with a syringe is not recommended, as it may increase the risk of contamination, iatrogenic spinal cord injury, and sometimes fatal brain herniation (SCOTT, 2005). The flow of CSF can be enhanced by applying digital pressure to the jugular vein (COLES, 1984; BRAUND, 1994; MCDONNELL, 1998; SCOTT, 2005). The complications that may occur during collection are described in Table 3.

Table 3: Complications observed during a cisternal and lumbar puncture

Complication	Cisternal Puncture	Lumbar Puncture
The needle touches the bone	The needle's out of the midline. You must put the needle back	The needle's out of the midline. You must put the needle back
CSF does not flow	The needle's out of the midline. There's spinal cord penetration, brain, or soft tissue damage.	The needle is out of the midline, the needle tip is in the spinal cord or there is soft tissue injury
CSF contaminated with arterial blood	Blood vessel puncture from the dura mater. Let it lighten or put the needle back in	Blood vessel puncture from the dura mater. Let it lighten or put the needle back in
Animal suddenly moves	Spinal cord damage. Remove the needle immediately	A muscle contraction during needle placement is normal.
Very strong CSF flow	Increased intracranial pressure. Remove the needle immediately	Increased intracranial pressure. Remove the needle immediately

Source: BRAUND, 1994; SCOTT, 1997; MCDONNELL, 1998; WHEELER & SHARP, 1999; TAYLOR, 2001; SCOTT, 2005

Small contamination of the CSF by blood does not interfere with its analysis or interpretation, but if the amount of blood is excessive, one should abandon the procedure and repeat it within 24 or 48 hours (BRAUND, 1994).

Laboratory Analysis - Macroscopic Examinations

There must be four sterile bottles available. The sample from the first tube should be used for biochemical and serological analyses. The second tube will be used for microbiological examinations, the third is for cell counts and the fourth will be used as a reserve if necessary (MELO & MARTINS et al., 2003).

The sample should be sent to the laboratory within sixty minutes of collection, because after this time there may be lysis or degeneration of the cells (RBCs, WBCs, and other cell types) since they are poor in protein and nucleated cells; there is also a decrease in glucose and an increase in the concentration of protein and bacteria after two hours. If it is not possible to perform the CSF analysis within the recommended period, the sample should be refrigerated between 2°C and 8°C (MCDONNELL, 1998; MELO & MARTINS et al., 2003; LUCAS & SACCO, 2008). Cooling the sample may slow cellular regeneration. If it is necessary to store the sample for two to four hours before analysis, it must be transferred to a plastic tube and an equal volume of bovine or patient serum must be added to slow cellular degeneration (TAYLOR, 2001).

The basic laboratory tests that should be performed are physical examination, glucose and protein analyses, and total and differential cell counts. If these tests reveal any abnormality, bacterial and/or fungal cultures may be performed, in addition to other biochemical analyses. (SARMENTO & TUDURY et al., 2000).

The volume required for most of the tests is 1 mL. If the sample is intended for microbiological examination or diagnosis of infectious disease, 2 mL should be collected. When collected for culture and/or identification, collect the sample in clotting type test tubes (MCDONNELL, 1998). If less than 0.5 mL of CSF is collected, preference should be given to more useful diagnostic tests, such as white blood cell and red blood cell counts, cytological examination and determination of protein concentration, in descending order of importance (MCDONNELL, 1998). The pathologist should be informed about the sampling site (SCOTT, 2005).

Physical/macroscopic examination

The normal CSF is clear, colorless (CHRISMAN, 1985; BRAUND, 1994; FENNER, 1998; WHEELER & SHARP, 1999; TAYLOR, 2001; MOURA et al., 2004; GAMA & SOBREIRA et al.,

2005) without clots, similar to distilled water (COLES, 1984) and the letters should be read easily through it (CHRISMAN, 1985; BROBST & BRYAN, 1989; DEWEY, 2003; PARRY, 2005).

Color and clarity can be easily assessed by comparing a tube of distilled water and a tube of CSF against a white surface (CHRISMAN, 1985).

Appearance changes

The CSF should be examined carefully, placing it against the light or in front of a text, in an environment with good luminosity so that changes in its turbidity can be noticed (Table 4) (SARMENTO & TUDURY et al., 2000; MELO & MARTINS et al., 2003). Turbidity usually happens when cells are present and do not become macroscopically evident until there are 500 or more cells/mL (COLES, 1984; BRAUND, 1994; GAMA & SOBREIRA et al., 2005; PARRY, 2005; LUCAS & SACCO, 2008).

Table 4: Changes in the appearance (turbidity) of the CSF and their possible causes

Appearance	Causes
Clear	Normal Liquor/Confirmation through Microscopic Examination
Slightly cloudy	Presence of blood cells, microorganisms or higher rates of protein and lipids
Cloudy	Increased presence of blood cells, microorganisms or higher rates of protein or lipids
Milky cloudy	Presence of numerous cells (leukocytes), bacteria, fungus and very high levels of protein or lipids

Source: COLES, 1984; BRAUND, 1994; SARMENTO & TUDURY et al., 2000; MELO MARTINS et al., 2003; GAMA & SOBREIRA et al., 2005; PARRY, 2005.

Color changes

The most common change in color is the appearance of a bright red fluid, resulting from the addition of fresh blood to the sample due to the rupture of blood vessels at the time of collection (COLES, 1984).

If the sample is pale pink or pale red, it should be investigated if such staining was caused by bleeding during collection or pathological lesions (CHRISMAN, 1985; SARMENTO & TUDURY et al., 2000; MOURA et al., 2004). If the bleeding was caused at the time of collection, when centrifuged, the sample will show a transparent supernatant in contrast to the xanthochromia supernatant that may appear if there is old bleeding (COLES, 1984; MOURA et al., 2004). Red cell contamination of the sample does not correlate significantly with nucleated cell count or protein concentration (SCOTT, 2005).

The xanthochromia CSF indicates old bleeding and may occur in trauma, vascular changes, and infections (CHRISMAN, 1985). Yellow staining of the supernatant after centrifugation is usually

caused by free bilirubin resulting from some old bleeding in the subarachnoid space (BRAUND, 1994). The most common causes of xanthochromia in the CSF include subarachnoid hemorrhage, myeloma hemorrhage, spinal block, neoplasia, acute inflammation, abscesses, and severe systemic jaundice (COLES, 1984; MOURA et al., 2004). Consider also the possibility of substances such as merthiolate, iodine, carotene, melanin, etc. (MELO & MARTINS et al., 2003).

A cloudy CSF is associated with infections, particularly bacterial infections, with an increased number of neutrophils (CHRISMAN, 1985). A greenish coloration of the CSF suggests a suppurative inflammatory process (BRAUND, 1994). A macroscopic discoloration (with increased turbidity) most often results from iatrogenic contamination with peripheral blood (PARRY, 2005).

Coagulation

The normal CSF does not coagulate (COLES, 1984; BROBST & BRYAN, 1989; PARRY, 2005). The coagulation will occur if there is an increase in fibrinogen if it is contaminated with large amounts of blood as a result of internal bleeding or contamination at the time of collection. Severe coagulation may occur in acute suppurative meningitis (COLES, 1984; PARRY, 2005).

Cell counts

Global Cell Count

For the total cell count, the undiluted CSF should be used, such count only deserves reliability when the material is clear, colorless, or when the number of cells is extremely low. Contamination of the CSF by blood makes effective testing difficult. (MELO & MARTINS et al., 2003). Normal values are variable according to the laboratory, but in general should be less than six leukocytes per microliter of canine CSF and less than two leukocytes per microliter of feline CSF (TAYLOR, 2001). Increases in these values are indicative of pathological processes and are called pleocytosis (FENNER, 1998; WHEELER & SHARP, 1999; PARRY, 2005). The relationship between the number of leukocytes found in microliters of CSF and its interpretation is described in Table 5.

The sample should be gently homogenized by inversion and the count should be carried out in a hemocytometer (Neubauer chamber) (WHEELER & SHARP, 1999; PARRY, 2005). The sample is applied on one side of the hemocytometer for the total cell count. The other side of the chamber is filled with CSF mixed with a dilution liquid, which is 0.1g of crystalline violet added to a 10mL mixture of glacial acetic acid and 90mL of distilled water (CHRISMAN, 1985; PARRY, 2005) and the solution is filtered before use to remove residues that may be confused with cells, in the proportion of 0.05mL of the dye to 0.5mL of CSF (PARRY, 2005). The dilution fluid smoothes the erythrocytes

and stains the leukocyte nucleus (CHRISMAN, 1985; PARRY, 2005). All cells should be counted in five of the nine square millimeter areas, multiplied by 2 on the undiluted side and by 2.2 on the diluted side; the number of cells on the undiluted side is equivalent to the leukocytes and erythrocytes, and the number of cells on the diluted side comprises only the leukocytes (CHRISMAN, 1985).

The normal CSF does not contain red blood cells (COLES, 1984; CHRISMAN, 1985; PARRY, 2005; LUCAS & SACCO, 2008) and if the sample is visibly contaminated with whole blood, a global red cell count may be indicated. In cloudy or bloody samples, the following RBC and WBC counting techniques should be used: For the RBC count, after homogenization, the material is diluted in a 1:10, 1:20, 1:50 or 1 test tube: 100, depending on the need, with Gower solution (composed of 50mL of reagent water and 16,5mL of pure acetic acid mixed, plus 6,25g of Anhydrous Sodium Sulfate, and making up to 100mL with reagent water), mix and fill both sides of the Neubauer chamber and count in the four outer quadrants and the central quadrant on both sides (10 quadrants). The result will be calculated by multiplying the number of cells found in the 10 quadrants by the dilution factor used and the result will be expressed in Haemias/microliters (MELO & MARTINS et al., 2003). The leukocyte count, in these cases, will be performed in the same way as the RBC count, but diluted in Turk's solution (composed of 50mL of reagent water and 2mL of pure acetic acid mixed, plus 1mL of Gentian violet 1% solution, and should complete the volume for 100mL with reagent water). The result will be calculated by multiplying the number of cells found in the 10 quadrants by the dilution factor used and the result will be expressed in Leukocytes/ microliter (MELO & MARTINS et al., 2003). If there are more than ten thousand erythrocytes per microliter of CSF it is preferable to repeat the analysis after 24 or 48 hours (BRAUND, 1994).

Table 5: Relationship between the number of leukocytes found/microliter of CSF and their interpretation

Number of Leukocytes/Microliter	Interpretation
5 to 10	Pathology suggestive
> 10	Confirmation of pathology
10 to 50	Mild inflammatory process, viral diseases, some trauma or vasculopathy
50 to 200	Moderate inflammation, fungus, protozoan and immune diseases
> 200	Acetous inflammatory process, bacterial meningitis or some immune diseases

Source: FENNER, 1998; WHEELER & SHARP, 1999; PARRY, 2005

Differential leukocyte count/cytological examination

The differentiation of cells should be done even if the total cell count is normal, since in such cases there may be the presence of abnormal cells such as eosinophils and tumor cells, as well as changes in cell proportions (WHEELER & SHARP, 1999; LUCAS & SACCO, 2008). For counting,

the leukocytes should be concentrated by centrifugation at 1500rpm for five minutes, or sedimentation, mounted on slides and stained (BROBST & BRYAN, 1989; WHEELER & SHARP, 1999). This procedure is necessary to obtain enough cells for cytological evaluation if the leukocyte count is less than 500 cells per microliter of CSF (TAYLOR, 2001; PARRY, 2005).

In a normal CSF strain, only small lymphocytes, few monocytes and rare neutrophils will be present, 95% to 100% of the cells being mononuclear, almost all corresponding to lymphocytes (COLES, 1984; CHRISMAN, 1985; BAILEY & HIGGINS, 1986; BROBST & BRYAN, 1989). A small number of macrophages can be seen (TAYLOR, 2001; PARRY, 2005). Occasionally, plasma cells of the choroid and ependymal plexus can be observed, and sometimes neutrophils and eosinophils are present, but these should not represent more than 10% of the total cell population (TAYLOR, 2001).

The blood contamination can significantly alter the differential leukocyte count of the CSF, increasing the number of neutrophils, and making it difficult to interpret the findings (TAYLOR, 2001).

Bacteria may occasionally be seen in the CSF. They can be pathogenic, but, if present in the absence of neutrophilic pleocytosis, they are more likely the result of contamination. Meningo-encephalitis granulomatosa is very rare in dogs and cats (WHEELER & SHARP, 1999). The observation of neutrophils is usually a sign of bacterial or pyogenic infection, or evidence of bleeding (COLES, 1984). Bacterial infections involving CSFs usually cause severe pleocytosis, largely because of neutrophils. An inflammation associated with viruses, fungus, neoplasms, or degenerative conditions commonly causes less drastic pleocytosis, with a higher proportion of lymphocytes. In response to inflammation of parasitic origin, eosinophils are generally observed, and the presence of neoplastic cells indicates that there is CNS neoplasia, but they are rarely found (COLES, 1984; PARRY, 2005). The relationship between the cells observed in the CSF and their interpretation are described in Table 6.

Table 6: Relationship between the cells observed in the CSF and their interpretation

Cells observed in the CSF	Interpretation
Small lymphocytes (mononuclear cells)	Normal
Severe Pleocytosis (Neutrophil Increase)	Bacterial infection, pyogenic infection (abscesses), non-infectious inflammatory conditions, hemorrhages, contamination by blood
Neoplastic Cells	Primary tumors involving CNS (mainly lymphoma or metastatic carcinoma)
Moderate pleocytosis (Increase in the number of primarily lymphocytic cells)	Viral infection/fungal infection, chronic infection, antibiotic therapies and corticotherapy, uremia
Eosinophilic Increased	Parasite inflammatory response/ Hypersensitivity, Myelography (foreign material)
Macrophages Increased	Viral infection, radiographic contrasts, presence of phagocytic red blood cells
Ependymary cells of the choroid plexus	Traumatism

Source: COLES, 1984; WHEELER & SHARP, 1999; PARRY, 2005.

Protein / total protein concentration

The normal CSF (of all species) has low protein concentration, usually 10 to 50mg/dL, being higher when collected by lumbar puncture, with almost all proteins corresponding to albumin (DEWEY, 2003; PARRY, 2005). This increase in protein content when the sample is collected by lumbar puncture is due to slow blood circulation in this area, leading to a local accumulation of protein (BROBST & BRYAN, 1989).

The protein concentration analysis is done to detect increased permeability of the blood-brain barrier and increased protein synthesis within the CNS (BROBST & BRYAN, 1989; SARMENTO & TUDURY et al., 2000).

CSF protein concentration can be measured qualitatively or quantitatively (PARRY, 2005). Quantitative methods are more precise (DEWEY, 2003).

The qualitative tests, Pandy and Nonne-Apelt tests, detect high molecular weight globulins (basically immunoglobulins), but are relatively insensitive tests, being considered only screening methods (SARMENTO & TUDURY et al., 2000; PARRY, 2005).

The Pandy test is simple and uses a solution prepared with 10 mg of crystals of phenic acid mixed with 150mL of distilled water; then a few drops of CSF are mixed to the solution and it is observed if turbidity occurs (SARMENTO & TUDURY et al., 2000; MOURA et al., 2004).

If the sample is normal there is no detectable immunoglobulin present and the solution remains clear (at most slightly cloudy), which is considered a negative result. (COLES, 1984; PARRY, 2005). If there is an immunoglobulin concentration of 25mg/dL or more, the solution will become cloudy-white. (PARRY, 2005). The degree of turbidity can be subjectively graded from 1+ to 4+, corresponding to the increasing immunoglobulin concentration (COLES, 1984; PARRY, 2005).

In the Nonne-Apelt test, 1 mL of saturated ammonium sulfate solution is carefully superimposed on 1 mL of CSF, without touching it for three minutes. The junction between the two fluids remains clear in the case of normal CSF. If the immunoglobulin concentration increases, a gray-white zone is formed at the junction (COLES, 1984; PARRY, 2005). This reaction can be subjectively graded from 1+ to 4+, reflecting the increased immunoglobulin concentration (PARRY, 2005).

As the protein concentration is like the protein concentration in urine, the urine strips can be used to estimate the protein concentration in the CSF (MCDONNELL, 1998). Most normal cats show a negative reaction (less than 30mg/dL) on the reagent strip (BRAUND, 1994). The most accurate and safe protein measurement is that taken with a spectrophotometer, the normal values being established by each laboratory (CHRISMAN, 1985).

The total protein may be increased in many disorders but is non-specific (WHEELER & SHARP, 1999). Affections known to increase CSF protein include encephalitis, meningitis, toxoplasmosis, neoplasms (mainly those located deep in the parenchyma of the brain), cerebrospinal fluid infarction, and trauma (COLES, 1984; FENNER, 1998; SARMENTO & TUDURY et al., 2000; MELO & MARTINS et al., 2003).

Some non-inflammatory states such as pneumonia, convulsive states, and uremia can alter capillary permeability, with the consequent appearance of globulins in the CSF (COLES, 1984). The amount of protein may appear altered in its total value, maintaining, however, its qualitative content unchanged, or it may increase in its absolute value with a parallel alteration in its qualitative content, that is, the appearance of globulins (SARMENTO & TUDURY et al., 2000). Globulins may be increased in the CSF due to hemorrhage. (COLES, 1984). Even a small hemorrhage causes significant protein increase (SARMENTO & TUDURY et al., 2000). Increased protein in the absence of pleocytosis is usually indicative of non-inflammatory disorder (WHEELER & SHARP, 1999).

Glucose Dosage - Glycorrhea

The normal CSF glucose concentration differs directly with the blood glucose concentration, almost always ranging from 60 to 80% of the blood value, therefore, to obtain a meaningful interpretation, the CSF and blood glucose concentration must be determined simultaneously (FENNER, 1998; PARRY, 2005).

The methodology used to evaluate glucose levels in the CSF is the same as that used for blood glucose determinations (PARRY, 2005). The increase in values is called hypoglycemia, while the decrease is called hypoglycemia (SARMENTO & TUDURY et al., 2000).

The measurement of glucose concentration is most useful as an aid in the diagnosis of acute suppurative septic inflammation involving the CSF; in this condition, the concentration of glucose in the CSF is much lower than 60% of the blood value, due to glucose metabolism by bacteria and neutrophils (PARRY, 2005). Reduced concentrations may occur in pyogenic infections such as bacterial meningitis (MOURA et al., 2004).

Hyperglycorrhea can be found in encephalitis, spinal cord compressions, abscesses, encephalic tumors, and hyperglycemic diseases such as diabetes mellitus (COLES, 1984).

Microbiological evaluation

Examination for the presence of bacteria can be carried out by microscopy of stained samples and culture (WHEELER & SHARP, 1999). If there are more than four cells per mm³ the microbiological analysis of the sample should be done (MOURA et al., 2004).

If bacterial or fungal changes are suspected, the CSF can be grown. Preparations of Gram stains and Indian ink from CSF cytology can demonstrate bacterial and fungal organisms respectively (CHRISMAN, 1984).

With the sample already centrifuged, an aliquot of sediment with platinum handle or Pasteur pipette should be taken and placed on a very clean microscope slide; 2 drops of china ink (India ink) are added and covered with a coverslip to homogenize the staining. This technique allows the visualization of *Cryptococcus neoformans* capsules, one of the most frequently found fungi in CSF samples. The dye used almost totally prevents the passage of light, except in those areas where there are structures, becoming, around these structures, lighter or more transparent concerning the microscopic field (SIDRIM et al., 1999).

Taylor (2001) states that in 60% of the samples sent to culture the *Cryptococcus neoformans* fungus causing cryptococcosis is isolated.

Changes observed in the analysis of the CSF in several disorders of dogs and cats

In the acute stage of distemper, there is no cellular inflammatory reaction and the total count and protein concentration in the CSF are normal. Then, during the disease, changes associated with an infiltration of mononuclear leukocytes and local production of immunoglobulins occur. More typically there is a medium to moderate pleocytosis characterized predominantly by lymphocytes. The total count is usually less than 25 cells/microliter. High cell counts, with a large percentage of neutrophils, have been reported in cases where there are areas of encephalomalacia associated with

the primary lesion. Protein concentration is moderately increased (greater than 50/mg/dL) and it is extremely rare to find inclusion corpuscles (MEIKOTH & CRYSTAL, 1999).

In cases of toxoplasmosis or neosporosis, mononuclear pleocytosis is observed with a predominance of lymphocytes and neutrophils corresponding to 10-20% of the cells present. A small percentage of eosinophils (5-10%) is not uncommon. The protein concentration is usually increased. Microorganisms are rarely observed in CSF samples (MEIKOTH & CRYSTAL, 1999).

Tumors in the CSF may alter the blood-brain barrier and increase the protein concentration in the CSF. Meningiomas are frequently in close contact with the subarachnoid space; an increase in leukocytes, predominantly neutrophils, can be observed with necrosis of these tumors (MCDONNELL, 1998). The findings depend on the anatomical location of the neoplasm; the type; the presence of necrosis of the tumor and/or adjacent tissue. Other types of tumors, especially metastatic neoplasms, sporadically have necrose-induced changes in the CSF that include marked pleocytosis, neutrophil predominance, and occasionally the degenerative appearance of neutrophils (MEIKOTH & CRYSTAL; 1999).

Feline Infectious Peritonitis (FIP) causes a huge response in proteins and cells. Meningitis or encephalomyelitis that occurs in FIP is typically observed in young cats under 3 years of age (MCDONNELL, 1998). In contrast to most viral infections, which produce moderate cell pleocytosis, the FIP virus is associated with a marked neutrophilic response (100-1000 cells/microliter) and protein concentration (greater than 100mg/dL) is markedly increased. The cell population that is usually described is pyogranulomatous, but in many cases, there are 75-95% non-degenerate neutrophils. In sporadic cases, there are predominant mononuclear cells (MEIKOTH & CRYSTAL, 1999).

Meningitis, bacterial, and steroid-responsive, should also be differentiated by the bacterial culture of the sample. In the latter, cultures will be negative, and hyper segmented or non-degenerate neutrophils will be observed (SARMENTO & TUDURY et al., 2000). CSF analysis in patients with steroid-responsive suppurative meningitis reveals a marked neutrophilic pleocytosis and a moderate increase in protein concentration. The total cell count is commonly greater than 100 cells/microliter and sometimes greater than 1000 cells/microliter. Non-degenerated neutrophils comprise 80% of the cells present and some changes or abnormalities in the cells may sometimes be present. A marked reduction in cell counts may occur due to steroid therapy (MEIKOTH & CRYSTAL, 1999).

In bacterial meningitis, there is a marked neutrophilic pleocytosis (greater than 1000 cells/microliter) and it is expected that a greater number of degenerated cells will be produced than

in deep cerebral parenchyma infections. The protein concentration may be increased in some cases (MEIKOTH & CRYSTAL.1999).

In patients with granulomatous meningoencephalitis, the results of CSF analysis are extremely variable and tend not to correlate with the histopathological findings of the same patient. A generalization of CSF findings is a mild to moderate pleocytosis characterized by a predominance of lymphocytes with 0-20% neutrophils and 0-20% monocytes/macrophages. Plasma and large, anaplastic and mononuclear cells can sometimes be seen. The total count may be markedly increased and approximately 10 to 15% of samples will be greater than 1000 cells/microliter. Although neutrophilic pleocytosis is more common, a true mixture of cells with pleocytosis (lymphocytes, monocytes, and neutrophils) occurs in many cases, with neutrophils making up more than 50% of the cells in rare cases. The protein is usually moderately increased (MEIKOTH & CRYSTAL.1999). It can be occasionally observed mitotic figures in the dog's encephalitis (COLES, 1984).

With the exception of cryptococcus neoforms that have a predilection for nervous tissue, positive results in CSF samples have been reported only sporadically for the various fungal diseases. Marked pleocytosis and increased protein concentration are characteristic. Pleocytosis is predominantly neutrophil (greater than 60%) and sometimes eosinophilic (MEIKOTH & CRYSTAL, 1999).

A few patients with ehrlichiosis have moderate mononuclear pleocytosis like viral infection (MEIKOTH & CRYSTAL, 1999). Patients with rabies virus may present lymphocytic pleocytosis, also observed in most viral infections (SARMENTO & TUDURY et al., 2000). Typical findings in CSF samples in several dog and cat conditions are summarized in Table 7.

Table 7: Typical CSF findings in the different conditions of dogs and cats

Disease	Total protein (mg/dL)	Total cell count (leucocytes/ μ L)	Differential leukocyte count
Hydrocephalus (congenital)	N	N	N
Hydrocephalus (postnatal)	+	+	Neutrophils
Aseptic meningitis	++/+++	++/+++	Neutrophils, Lymphocytes
Intervertebral disc protrusion	N or +	N	N
Corticosteroid-responsive meningitis	+++	+++	Mature non-toxic neutrophils
Meningeal Vasculitis	+++	+++	Mature non-toxic neutrophils
Meningoencephalitis granulomatosis	++	++	Lymphocytes, Monocytes, sometimes
Bacterial meningitis	+++	+++	Plasmocytes. Neutrophils in 60% (>20% of the
			Toxic Neutrophils, Bacteria
			Small lymphocytes
Cinomose	+	+	
Toxoplasmosis	++	++	Lymphocytes, Macrophages, occasionally PMN
Neosporosis	++	++	Lymphocytes, Macrophages, occasionally PMN
Cryptococcosis	++	++	Lymphocytes, Macrophages, occasionally PMN and neutrophils
Ehrlichiosis	+	++	Lymphocytes
Feline Infectious Peritonitis (FIP)	+++	+++	Mixed mononuclear neutrophils
Migration of parasites by CNS	+	+	Monocytes, occasionally eosinophils, Neutrophils
Spinal cord or cerebral infarction	+	N or +	Mononuclear cells, Neutrophils
CNS neoplasms (except lymphoma)	+	N or +	Mononuclear cells
Meningioma	++	N or ++	Mononuclear cells, Neutrophils
Lymphoma in the CNS	+	++	Lymphocytes, Neoplastic Cells
Lissencephaly	N	N	N
Polyradiculoneuritis	N or +	N	N
Degenerative myelopathy	N or +	N	N

PMN = polymorphonuclear neutrophils

N = Normal

+ = slight increase

++ = moderate increase

+++ = marked increase

Leukocyte count (leuc/ μ L)

N= Normal

+ = <50

++ = 50-100

+++ = >100

Protein (mg/dL)

N= Normal

+ = 25-50

++ = 50-100

+++ = >100

Source: COLES, 1984; MCDONNELL, 1998; MEIKOTH & CRYSTAL. 1999; SARMENTO & TUDURY et al., 2000

2 CONCLUSION

The Laboratory of Clinical Pathology is essential in the veterinary clinic since the results obtained in the laboratory analyses help the veterinary doctors to establish diagnostics of the several diseases, to have an overview of the clinical condition of the patient, besides indicating the prognostic for each patient in individual.

The collection, analysis, and interpretation of the CSF of small animals, when performed correctly, serve as auxiliary methods in the investigation of diverse neurological diseases, representing one of the best methods for the diagnosis of brain and spinal cord disorders.

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