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Review

The function, composition and analysis of cerebrospinal fluid in companion animals: Part II – Analysis

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Abstract

Accurate analysis of cerebrospinal fluid (CSF) provides a wide range of information about the neurological health of the patient. CSF can be withdrawn from either of two cisterns in dogs and cats using relatively safe techniques. Once CSF has been collected it must be analysed immediately and methodically. Evaluation should consist of macroscopic, quantitative and microscopic analyses. As part of a quantitative analysis, cell counts and infectious disease testing are the most important and potentially sensitive indicators of disease. Although certain pathologies can be described, microscopic analysis will rarely be specific for any disease, emphasising the adjunctive nature of this diagnostic modality.
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Keywords: Cerebrospinal fluid; Analysis; Dog; Cat; Neurological disease

Introduction

Accurate analysis of cerebrospinal fluid (CSF) provides a wide range of information about the neurological health of a patient. Similar to a complete blood count, CSF has high sensitivity but low specificity for the detection of disease. The possible abnormalities of CSF are relatively limited given the varieties of neurological disease that exist. CSF analysis is not always abnormal with neurological diseases (Braund, 2003) but occasionally it will help to provide a specific diagnosis. For these reasons, accurate anamnesis, physical and neurological examinations, imaging studies and other diagnostic tests are essential for an accurate and correct interpretation of CSF changes in an individual case (Chrisman, 1992).

The first part of this paper discussed the function and composition of CSF (Di Terlizzi et al., 2006). This review will address the collection, sample processing and complete analysis of CSF in companion animals.

CSF collection

CSF analysis may be useful as an important component of the diagnostic evaluation of patients with central and peripheral neurological disease. CSF should be collected wherever an inflammatory, infectious, traumatic, neoplastic or degenerative disorder of the brain and the spinal cord is suspected. However, the analysis of CSF may only support the diagnosis of a central nervous system (CNS) disorder and is rarely definitively diagnostic.

CSF can be collected from the cerebellomedullary cistern (CMC) or the caudal lumbar subarachnoid space. Because the fluid flows predominantly in a rostro-caudal direction, it is more diagnostic and therefore preferable to collect it from a site caudal to the suspected lesion (Thomson et al., 1990). Collection of CSF requires the patient to be under general anaesthesia with the site of collection clipped and aseptically prepared. No more than

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1 mL of CSF per 5 kg bodyweight (in dogs, cats and horses) should be collected (Carmichael, 1998). The equipment required for sampling includes sterile plain collection tubes (polymerase chain reaction [PCR], will require EDTA tubes), 20–22 G 40–90 mm (1.5–3.5 in.) spinal needles, and sterile surgical gloves. Small hypodermic needles for CSF collection (22 or 25 G) may be useful in small dogs and cats and are safer if the operator is inexperienced in assessing the depth of the relative cisterns from the skin surface. In such patients there may not be an obvious 'sensation' that accompanies penetration of the dura; a needle without a stylette, such as a hypodermic needle, will help the user identify the time of puncture of the dura with a 'flash' of CSF that simultaneously appears in the hub. A spinal needle may be used, removing the stylette after piercing the skin, but the larger size needle makes the procedure cumbersome in a small dog.

Risks and contraindications

General anaesthesia is required for CSF collection, and anaesthesia needs to be considered as a risk for every patient with intracranial disease. A specific risk of CSF collection is iatrogenic brainstem trauma or spinal cord trauma due to needle puncture (Platt et al., 2005; Lujan Feliu-Pascual et al., 2006). An aseptic technique is required because of the potential for introducing infectious agents to the CNS (Cook and DeNicola, 1988).

A frequently documented specific contraindication to CSF collection is that of increased intracranial pressure (ICP). Increased ICP is generally a non-specific finding and may be associated with space occupying lesions, cerebral trauma, hydrocephalus, and inflammatory CNS diseases (Braund, 2003). Normal values of pressure are <170–180 mm H_2O for dogs and <100 mm H_2O in cats (De Lahunta, 1983; Braund, 1986; Oliver and Lorenz, 2005). However, practically, ICP and associated cerebral perfusion pressures are rarely measured and so reliance is placed upon clinical signs suggestive of increased ICP.

The neurological signs associated with increased ICP are unfortunately non-specific and include altered mentation (depression progressing to stupor and coma), paresis, poor pupillary response to light, abnormal and/or unequal pupil size, decerebrate and decerebellate rigidity and vertical nystagmus (Smith and Madsen, 2004). Some of these signs may only appear terminally as a consequence of brain herniation. Caudal transtentorial herniation results in pressure exerted downward through the midbrain with subsequent compression of the oculomotor nerve nucleus resulting in pupil dilation and poor response to light (Kornegay et al., 1983). With unilateral herniation, an ipsilateral dilated pupil, unresponsive to light stimulation, may be seen. These signs may be too unreliable to assist with the detection of increased ICP and subsequent herniation as oculomotor palsy specifically occurred in only 14% of 54 dogs with brain herniation (Walmsley et al., 2006).

Increased ICP may sometimes be associated with systemic manifestations of increased blood pressure and decreased heart rate as part of the so-called Cushing's response (Jones, 1989). In dogs and cats, heart rate and blood pressure may be more accurate indices of a sudden change in ICP. Suggested heart rates <60 and blood pressures of >180 mm Hg may indicate elevated ICP in people, but there is currently no evidence for this in veterinary medicine. In patients with elevated ICP, a needle inserted in the subarachnoid space in either the cisterna magna or the lumbosacral space may produce a pressure gradient sufficient to cause a caudal shift of intracranial structures (herniation) (Evans, 1988; Rand et al., 1994b). If there are clinical signs suggestive of elevated ICP, and advanced imaging is not available prior to CSF collection, consideration should be given to pre-administration of an osmotic diuretic such as intravenous mannitol. Further aggressive treatment may include hyperventilation but it is essential to keep the patient's pCO_2 level above 35 mm Hg to avoid vasoconstriction induced ischaemia. The overall risk of cisternal puncture in patients with underlying intracranial disease has not been documented but is low in the authors' experience.

Other contraindications to CSF collection include the suspicion of an underlying coagulopathy, atlanto-axial sub-luxation, Chiari-like malformation or cervical trauma. As with any procedure the risk and benefit of CSF collection should be considered in each case.

Technique

CMC collection

Patients are positioned in lateral recumbency, with the skull and cervical vertebrae at the edge of the table, and the skull fully flexed to create a 90° angle with the cervical spine. A reinforced endotracheal tube may be used to reduce the chance of 'kinking', and the cuff may be deflated at the point of maximum neck flexion to reduce the chance of tracheal trauma. The nose is slightly elevated in order to position the long axis of the muzzle parallel to the table. The entry site for the spinal needle is at the intersection of imaginary lines drawn from the occipital protuberance to the dorsal arch of C2 horizontally and along the rostral aspect of the wings of the atlas vertically. The needle should be kept perpendicular to the dorsal laminae of the vertebral column, at the level of the atlanto-occipital space, and advanced very slowly through the skin (Fig. 1).

Other methods of determining the site of puncture have been described, but the aforementioned method is preferred by the authors. As it is advanced, resistance may be felt just before the needle pierces through the atlanto-occipital ligament, the meninges (dura mater and arachnoid) and into the cisterna magna. The stylette of the spinal needle is removed at this point, and the fluid is allowed to flow into plain sterile collection tubes. Many times this resistance is not felt, particularly in small dogs and cats.

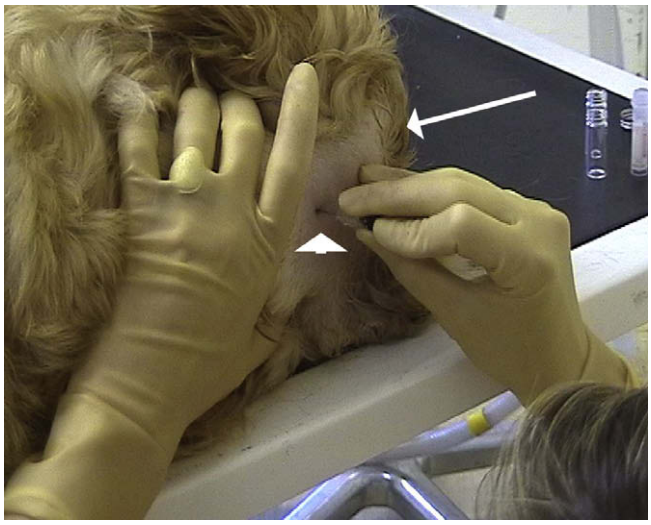

Fig. 1. CSF collection from the cerebro-medullary cistern (CMC). The dog is in right lateral recumbency with the head to the right of the picture (arrow). Gloves are worn by the operator when handling the needle. The operator's left hand then palpates the wings of the first cervical vertebra, whilst the right hand simultaneously palpates the occipital protuberance and carefully inserts the needle. The insertion point is at the intersection of a vertical line delineating the cranial aspect of the wings of C1 (arrowhead) and a horizontal line joining the occipital protuberance and the dorsal arch of C2.

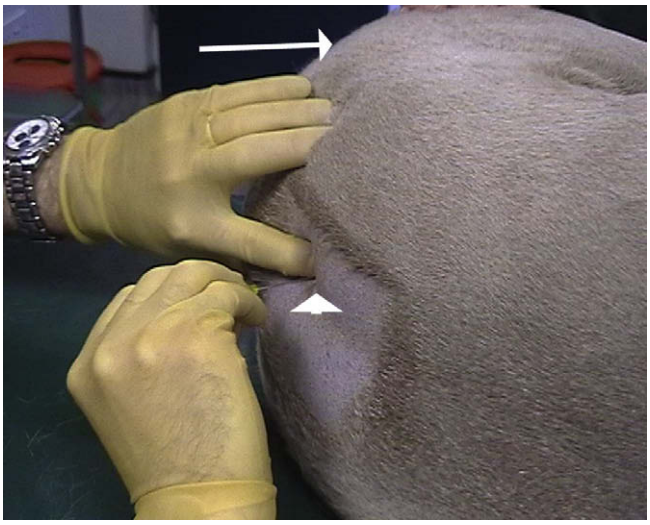

Fig. 2. CSF collection from the lumbar cistern. The dog is in right lateral recumbency with the caudal end indicated by the arrow. A sterile area is prepared over the L5 to S2 vertebrae as shown. Sterile gloves are worn to handle the needle but drapes are not required to ensure sterility. The insertion point is at the cranial aspect of the dorsal spinous process of L6 (arrowhead) and the needle is inserted perpendicular to the spinal column.

To prevent cord damage, reliance should not be placed on the presence of this sensation and, instead, either the stylette can be withdrawn once the needle is in the muscle or a hypodermic needle can be used. In each case, CSF will 'flash' back into the hub as soon as the cisterna is entered. If the needle hits bone during its passage, the needle may be slightly withdrawn and redirected more caudally. If blood appears in the hub of the needle, the needle should be withdrawn at once and the procedure repeated following reassessment of the anatomical landmarks.

Generally, 0.75–2 mL fluid is sufficient for protein and cellular examinations (Cook and DeNicola, 1988). In the authors' experience, most laboratories can comfortably assess protein levels, cytology and cell counts when supplied with 0.5 mL of CSF. A few drops should be saved in separate plain tubes for microbial culture and sensitivity if infection is suspected, and for virological and immunological studies, if needed. An EDTA tube is necessary if the sample is for PCR analysis. Once collection is completed, the needle is gently removed from the site of collection, and if further fluid is required, the needle should be placed over the collection tube as it empties out its contents.

Lumbar collection

Technically, lumbar collection is more difficult to perform than the CMC collection and more likely to result in iatrogenic blood contamination. The patient is positioned in lateral recumbency, with the pelvic limbs fully flexed (Fig. 2). The appropriate intervertebral space is L5–L6 in dogs and L6–L7 in cats (Oliver and Lorenz, 1997). At these spaces, the spinal cord has tapered into the conus medullaris and is surrounded by nerve roots or the cauda equina, which are much less likely to be damaged by needle insertion than the cord itself. The subarachnoid space rarely extends to the lumbosacral junction in dogs (Oliver et al., 1987), whereas collection may sometimes be made from the lumbosacral space in cats.

The needle is inserted just caudal to the space of interest, perpendicular to the dorsal laminae of the vertebrae, along the cranial border of the caudal dorsal spinous process. If the needle hits bone, it should be moved a few millimetres cranially or caudally. In most medium to large sized dogs, it is necessary to use 20 G needles to reduce needle bending following the necessary manipulation. A slight twitch of the tail or leg may occur upon insertion of the needle inside the canal due to stimulation of the nerve roots or cauda equina following irritation or penetration by the needle. The needle is often inserted until it contacts the bone of the ventral aspect of the spinal canal; the stylette is then removed and the fluid collected as above. If no fluid appears, very slight rotation and/or withdrawal of the needle should encourage CSF flow. The rate of fluid flow is usually slower than from the CMC, and the fluid quantity retrieved less.

Sample processing

CSF samples should be collected in sterile plain tubes. EDTA tubes are not recommended for routine sampling, as the additive can falsely elevate the total protein concentration (Parent and Rand, 1994).

A significant distortion of cell structure and reduction of total nucleated cell count (TNCC) from cell lysis may occur if processing is delayed (Fry et al., 2006). This fragility is

partly related to the typically low CSF protein levels, lipid concentration and tonicity, factors which in other fluids contribute to stabilisation of cell membranes (Steele et al., 1986). If processing is delayed for longer than 1 h, cellular changes occur, which include nuclear pyknosis, lysis and disintegration of the cytoplasmic and nuclear membranes. At room temperature, neutrophils degenerate most rapidly (within 1 h) and lymphocytes and monocytes start to degenerate after 3 h (Kjeldsberg and Knight, 1986). However, a recent study on CSF stored at 4 °C without additives and analysed at 2, 4, 8, 12, 24, and 48 h demonstrated that small mononuclear cells deteriorate most rapidly, followed by large mononuclear cells, neutrophils and then eosinophils (Fry et al., 2006). Eosinophil structure was frequently unchanged after 48 h of storage. Cells of the same type started to degenerate with considerable terminal variation, and after 48 h cells tended to clump or adhere to each other (Fry et al., 2006).

Delaying analysis of canine CSF by 4–8 h is unlikely to alter diagnostic interpretation if the protein concentration is $\geqslant$50 mg/dL (Fry et al., 2006). Therefore, addition of fetal calf serum (FCS) with a protein concentration of 3.7 g/dL (Biuret method) to CSF at a concentration of 20% vol, or the addition of hetastarch to CSF at a ratio of 1:1 (vol:vol) can help to stabilise cells in CSF (Fry et al., 2006). FCS appears to stabilise mononuclear cells more effectively than hetastarch 48 h after collection (Fry et al., 2006). The addition of 1 drop of 10% formalin to 1–2 mL of CSF may be used to preserve cell concentration and structure for up to 8 h after collection (Evans, 1988; Carmichael, 1998). Alternatively, cellular stability can be increased for up to 24 h from the time of collection by addition of fresh or frozen autologous clear plasma or serum (11% by volume) (Bienzle et al., 2000).

If the analysis requires a delay, two aliquots of CSF can be collected and placed in sterile plain tubes. In one of the tubes, a preservative should be used and the sample can be submitted for cell counts and cytological evaluations. The sample without preservative can be used for protein quantification and antibody titre analysis.

Macroscopic evaluation

Colour

Normal CSF is colourless. Any change in colour generally represents an abnormality. Pink or red colouration suggests the presence of blood. If, after centrifugation, a red cellular pellet is present at the bottom of the tube and the supernatant is colourless, the colouration was due to the presence of intact erythrocytes from iatrogenic peripheral blood contamination or recent (few hours) haemorrhage in the subarachnoid space (Cook and DeNicola, 1988). If the supernatant is xanthochromic (yellow to yellow-orange discolouration), previous haemorrhage and accumulation of oxyhaemoglobin or methaemoglobin derived from erythrocyte degradation is likely (Kjeldsberg and Knight, 1986; Cook and DeNicola, 1988). The intensity of the colour peaks 24 h after haemorrhage and disappears by 4–8 days (Jamison and Lumsden, 1988). Xanthochromia has also been reported when there is an increase in total protein concentration, hyperbilirubinaemia, and also with CNS inflammation and neoplasia (Krieg, 1979; Cook and DeNicola, 1988) (Table 1).

Turbidity

Normal CSF is translucent. An increased turbidity is attributed to particles in the fluid and typically is due to increased cellularity of the sample. Mild to moderate elevations in TNCC rarely alter CSF clarity (Chrisman, 1992), whereas a nucleated cell count of >500 cells/μL is associated with an increase of turbidity (Coles, 1986).

Quantitative analysis

Protein concentration and total nucleated cell count

CSF has an extremely low protein concentration relative to serum (Fishman, 1992). In dogs and cats, as well as in people, the total protein concentration increases along the neuraxis rostro-caudally, and for this reason lumbar fluid typically has a higher protein and lower nucleated cell concentration when compared with the CMC fluid (Bailey and Higgins, 1985). The reasons for the difference in cell concentration are not well understood: the lower cell concentration may result from increased cell lysis as the CSF flows in a caudal direction (Fishman and Chan, 1980). Higher protein concentration is also thought to arise from the slower circulation of CSF in the lumbar region with subsequent local protein accumulation (Thomson et al., 1990). Other studies suggest that the increasing concentration of protein from the ventricles to the CMC, and from the CMC to the lumbar region is due to the higher permeability of the blood–CSF barrier to proteins in the lumbar region (Fishman et al., 1958) (Table 2).

Total protein concentration

Almost all of the proteins normally present in CSF are derived from plasma (Reiber, 1998; Reiber, 2003). In normal CSF, protein levels consist almost entirely of albumin. There are minor concentrations of transthyretin (TTR or prealbumin), retinol-binding protein (RBP) and transferrin, which are synthesised by the choroid plexi (Aldred et al., 1995); in addition, there are traces of beta and gamma globulins, tau protein (a fraction of modified transferrin), glial fibrillary acidic protein, and myelin basic protein, which appear to be synthesised intrathecally (Thompson, 1988). There are quantitative and qualitative differences between plasma and CSF TTR and RBP in dogs, suggestive of a selective and controlled transfer of retinol into CSF and a local synthesis of TTR and RBP in the choroid plexus (Forterre et al., 2006).

Reference intervals for CSF total protein concentration can vary with the laboratory and testing method used. Refractometer evaluation is not accurate for the measurement of CSF total protein concentration (Chrisman, 1992). Urine protein reagent strips may be useful for an initial preliminary screening test to estimate CSF protein concentration, however it is highly specific for albumin detection and less specific for globulin detection, only providing a crude quantification (Jacobs et al., 1990). False-positive and false-negative test results may occur at dipstick readings of trace or 1+. However, dipstick readings of 2+ or above reliably represent a true increase of total protein concentration (Jacobs et al., 1990). The Pandy test is a screening test for the presence of globulins. It is performed by adding a few drops of CSF to 1 mL of Pandy reagent (10% carboxylic acid). Turbidity indicates the presence of globulin and has a sensitivity of approximately 50 mg/dL or 0.5 g/L.

For an accurate evaluation of CSF protein concentration, special analytical techniques are necessary (Chrisman, 1992; Parent and Rand, 1994). The most common protein assays use the specific dyes, Coomassie blue and pyrogallol red (Marshall and Williams, 2000). Pyrogallol red is considered the most specific technique for CSF total protein determination (Marshall and Williams, 2000), but it underestimates CSF total protein in dogs due to 20% lower affinity for globulins than for albumins (Behr et al., 2003). A human immunoturbidimetric assay (microalbumin) has recently been validated to measure canine albumin concentration in urine and CSF (Gentilini et al., 2005).

Albumin and albumin quotient

The major protein in the CSF is albumin (80–95%), which is only synthesised in the liver (Evans, 1988). The concentration of CSF albumin is much lower than the concentration of serum albumin, but the ratio is kept constant in healthy states; for this reason the calculation of the ratio between CSF and serum albumin, called albumin quotient (AQ), may be useful to evaluate a disruption of the blood-brain barrier (BBB). A thorough discussion of AQ and its calculations and interpretations has been given elsewhere (Sorjonen, 1987).

Gamma-globulins and IgG index

Electrophoretic techniques define the gamma-globulins as a heterogeneous group of proteins with similar migration rates. The gamma-globulin fraction contains the immunoglobulins (Ig). Three major immunoglobulins are found in CSF: IgG, IgM, and IgA (Bailey and Vernau, 1997). The major immunoglobulin in normal CSF is IgG, which normally originates from the plasma. Small amounts of IgG are usually found in the CSF of normal dogs and cats (Bailey and Vernau, 1997). Increased IgG levels may occur in a number of inflammatory CNS disorders. In these conditions, gamma globulin may enter the CSF through dysfunctional blood–brain/CSF barriers, or be synthesised intrathecally due to the local disease process (Tipold et al., 1994).

Serum IgG concentrations are at least 1000× higher than those in the CSF, but there is a strong association between the two values. A ratio of the values can be more useful than the absolute CSF IgG concentration alone (Tipold et al., 1993). IgG ratio and IgG index equations and their interpretation have been already described in previous reviews (Bichsel et al., 1984; Vandevelde et al., 1986; Tipold et al., 1993; Tipold, 1995b). Based on the authors' experience, these calculations are usually reserved for specific cases where an infectious disease is suspected.

In addition to the IgG index, recent studies have described other techniques to measure intrathecal antibody production: the *Antibody index* (Knopf et al., 1998) and another similar technique called the *Goldman–Witmer coefficient (C-value)* (Potasman et al., 1988). Both techniques are believed to be more accurate than the IgG index, as they use antigen-specific antibody titres rather than total IgG (Furr, 2002).

The presence of IgM is not a normal finding in the CSF and its presence can have diagnostic significance (Krakowka et al., 1981). IgM is detected in the earliest stage of the general humoral immune response of the body and it is the first immunoglobulin that returns to normal

Table 1
Component of CSF evaluation necessary in all routine analyses and commonly cited reference intervals (see text)

Macroscopic evaluation	CSF reference intervals
Colour	Colourless
Turbidity	Clear
Erythrocytes	0 to small number
Total nucleated cell count	0–5 cells/μL (dog) 0–8 cells/μL (cat)
Total protein	<30 mg/dL (cerebellomedullary) <45 mg/dL (lumbar cistern)
Microscopic evaluation	
Differential cell count	Lymphocytes and monocytes predominate, few segmented non-degenerate neutrophils, and rare erythrocytes

Table 2
Other CSF tests that might be performed in particular conditions (see text)

Test	CSF reference intervals	Condition
Albumin quotient (AQ)	<2.35	↑ BBB permeability
Immunoglobulin G index (IgG)	<0.272 with normal AQ	Intrathecal IgG
Antibody index	Negative	Specific antibodies
IgA	Negative	SRMA
Protein electrophoresis	N/A	Intrathecal IgG
Flow cytometry	N/A	Immunophenotyping
Myelin basic protein	Negative	Demyelinating disease
CSF culture	Negative	Infectious diseases
PCR organisms	Negative	Infectious diseases
Matrix metalloproteinase	Negative	Research analyte

SRMA, steroid-responsive meningitis–arteritis; BBB, blood-brain barrier; CSF, cerebrospinal fluid; PCR, polymerase chain reaction.

concentration when the antigen disappears. The presence of IgM in serum and/or CSF is considered more specific than IgG or total immunoglobulin levels for detection of active infectious diseases (Chrisman, 1992).

IgA is normally present in low concentrations in serum, but is involved as a major first-line defensive role in infections that enter via mucosal surfaces. The highest concentrations of intrathecal as well as systemic IgA have been found in dogs with steroid responsive meningitis–arteritis (SRMA) (Tipold, 1995a) and so are used as an adjunctive diagnostic procedure for this condition. In this instance, IgA appears to play a central role in the CNS humoral response (Tipold et al., 1994). Unfortunately, increased IgA levels in CSF can also be found in other inflammatory diseases such as granulomatous meningoencephalomyelitis and canine distemper encephalitis (Tipold, 1995a). Based on our experience, IgA levels in the CSF should be routinely measured in suspected cases of SRMA and is clinically useful.

Other proteins which may be measured in the CSF include myelin basic protein (Summers et al., 1987; Oji et al., 2007), S-100 and C-reactive protein, the latter of which may help to differentiate bacterial from viral meningoencephalitis (Fishman, 1992; Stearman and Southgate, 1994). Myelin basic protein (MBP) was studied by some authors (Oji et al., 2007) who found an increase in MBP concentration in CSF collected from the lumbar cistern in dogs with degenerative myelopathy, but not in the CSF collected from the cisterna magna or from either site in control dogs. These findings suggested that an active demyelinating lesion in the spinal cord was present. Also, the same study confirmed the correlation between MBP and the severity of the demyelination (Oji et al., 2007). These tests currently have limited availability, and remain of academic interest in veterinary medicine.

Cell count

The cellular concentration of normal and sometimes abnormal CSF is too low to be detected by standard haematological analysis. For this reason, cell counts are performed using a haemocytometer (Neubauer chamber or Fuchs-Rosenthal chamber). The chamber is placed in a humidified environment (e.g., Petri dish with damp tissue) for up to 10–15 min to allow cells to settle to the surface of the glass. The erythrocytes and the nucleated cells are counted separately. In the Neubauer chamber, the total cells present in all nine large squares of the chamber are counted. The calculations are performed using the average of the two sets of nine squares:

$$\text{Number of cells} \times 10/9 = \text{cells}/\mu\text{L}, \text{cells}/\text{mm}^3 \text{ or cells} \times 10^6/\text{L}.$$

In the Fuchs-Rosenthal chamber the cell the total cells present in 16×1 mm^2 areas are counted and the following formula is applied:

$$\text{Number of cells} \times 10/9 = \text{cells}/\mu\text{L}, \text{cells}/\text{mm}^3 \text{ or cells} \times 10^6/\text{L}.$$

In this case, the cells are counted and divided by 3 instead of 3.2 and the 10/9 dilution is disregarded. The two fractions will compensate for each other so that the remaining error will be negligible.

Normal CSF should not contain erythrocytes, but they may be seen in low numbers due to iatrogenic blood contamination. Erythrocytes can also be seen associated with pathological acute haemorrhage. Practice is needed to differentiate RBCs from WBCs in the haemocytometer chamber using phase microscopy, especially small mature lymphocytes because of their similar size. In general, RBCs lack a nucleus and internal structure, while WBCs are larger and have a granular appearance. Sometimes crenation of RBCs can be helpful in their identification (Meinkoth and Crystal, 1999). When an unstained specimen is examined, it is necessary to lower the microscope condenser to reduce the light intensity. Alternatively, the cells may be stained with a small amount of new methylene blue stain whilst in a microhaematocrit tube, and the stained CSF can be drawn into the haemocytometer chamber (Meinkoth and Crystal, 1999).

Abnormalities in cell type or morphology may be present even when CSF nucleated cell count are within normal limits (Christopher et al., 1988). In many disease processes, the CSF cell count and CSF total protein concentration tend to increase in parallel (Carmichael, 1998). In some disorders the cell count remains normal, whereas the total protein concentration may be markedly increased; this condition is called albuminocytological dissociation or protein-cytological dissociation (Evans, 1988; Carmichael, 1998).

Elevated CSF protein concentrations without increases in CSF nucleated cell count have been described in viral non-suppurative encephalomyelitis (Bichsel et al., 1984; Sorjonen, 1987; Sorjonen, 1990), neoplastic disease, traumatic, vascular, degenerative, and compressive spinal cord lesions (Evans, 1988; Chrisman, 1992).

Other tests

Antibody titres

CSF infectious disease titres may be more reliable than serum titres (Matsuki et al., 2004). A variety of antibody and antigen tests are available for detecting viral, fungal, protozoal, and rickettsial agents (Matsuki et al., 2004). Interpretation of CSF antibody titres, when performed in isolation, may not be accurate, because intrathecal antibody production can be difficult to differentiate from a disruption of the blood brain barrier or false elevation of serum antibodies due to blood contamination (Bailey and Vernau, 1997).

Intrathecal production of antigen-specific antibody can be determined with an antibody index in the same way as

intrathecal IgG production is detected with the IgG index (Reiber and Lange, 1991). Antibody indices have been calculated in human patients with a variety of diseases (Reiber and Lange, 1991), but they need further study for application in veterinary medicine.

The prevalence of autoantibodies in the CSF from dogs with various CNS diseases has recently been documented (Matsuki et al., 2004). Anti-astrocytic autoantibodies in canine CSF were detected and considered highly specific for necrotising meningoencephalitis (NME) and granulomatous meningoencephalomyelitis (GME); however, anti-astrocytic autoantibodies have also been detected in cases of brain tumours (Matsuki et al., 2004).

Recent studies (Boettcher et al., 2007) demonstrated that diseases associated with severe vasculitis like FIP may disrupt and impair the blood–brain barrier and CSF flow rate. In those cases, the measurement of the CSF antibodies may be increased due to increased vascular permeability, therefore reflecting the concentration of antibodies in the plasma rather than estimating the intrathecal production of antibodies. The calculation of AQ and specific antibodies index may be useful in these cases as an indicator of intrathecal antibody production (Boettcher et al., 2007).

CSF culture

When an infectious organism is suspected to be the cause of a CNS disease, both aerobic and anaerobic bacterial cultures of CSF may be performed. However, positive bacterial culture results in confirmed cases of bacterial meningitis are extremely uncommon (Tipold, 1995a; Fenner, 1998; Radaelli and Platt, 2002). A negative culture may be the result of inappropriate sample handling or culture media, in addition to a low number of organisms in the CSF (Fenner, 1998; Garges et al., 2006). Additionally, some bacteria are prone to rapid autolysis in collection tubes (Peters et al., 1995). PCR techniques have been used to detect the presence of bacterial DNA in people (Peters et al., 1995), but are not routinely used in veterinary medicine. Fungal culture of CSF can be used to isolate *Cryptococcus* spp. and virus isolation has also been successful in diagnosing cases of distemper meningoencephalitis (Kai et al., 1993; Fenner, 1998).

Polymerase chain reaction (PCR)

PCR can identify an infectious agent's DNA or RNA, and is extremely useful when the organisms cannot be cultured (Sharp, 1998). Single-round PCR amplification is used for the majority of infectious agent's such as canine distemper virus (CDV), *Toxoplasma gondii*, *Neospora caninum*, *Ehrlichia canis*, *Rickettsia rickettsii*, *Bartonella* spp., *Borrelia burgdorferi*, feline leukaemia virus (FeLV), and feline immunodeficiency virus (Stiles et al., 1996).

Some studies have shown that a multiplex PCR assay with built-in control reactions may be useful as a complementary clinical tool for the ante-mortem diagnosis of toxoplasmosis or neosporosis associated with the CNS (Schatzberg et al., 2003). Reverse transcription-PCR (RT-PCR) is used to amplify the RNA template nucleoprotein of canine distemper virus (CDV) in the CSF (Frisk et al., 1999). Reverse transcriptase-PCR represents a sensitive and specific method for an ante-mortem diagnosis of CDV if serum or whole blood are examined at the same time (Frisk et al., 1999). In our experience, however, a negative PCR assay does not rule out the presence of the infectious agent.

Research analyses

The following analyses are currently not clinically evaluated and as such are research tools but their potential utility is discussed.

Matrix metalloproteinases (MMPs)

MMPs form a large group of zinc-dependent endoproteinases (endopeptidases) that are able to degrade components of the extracellular matrix (ECM) (Birkedal-Hansen et al., 1993). Matrix MPs can be divided into four groups based on substrate specificity: collagenases, stromelysin, gelatinases, and membrane-type MMPs. The ECM is important in maintaining the structure of the CNS in addition to its role in transport of ions, cell migration and delivery of growth factors (Perides et al., 1998). MMPs have several roles in wound healing, angiogenesis, and embryologic development. They are also involved in neuropathological processes such as tumour migration, axonal degeneration, lumbar disk herniation, multiple sclerosis and acute spinal cord injury (Matsui et al., 1998).

MMPs have been characterised in dogs. Specifically, the enzyme activities of MMP2 and MMP9 have been found in a variety of tissues (Lana et al., 2000) including synovial fluid, brain, myocardium as well as osteosarcomas and mast cell tumours in dogs (Gilbert et al., 1997; Coughlan et al., 1998; Leibman et al., 2000). In people, the proenzyme form of MMP (proMMP)-2 has been found in normal brain and CSF, whereas MMP-9 has been found only during pathological processes (Mun-Bryce and Rosenberg, 1998). Many cells in the CNS produce MMPs (neurons, microglia, oligodendrocytes, and astrocytes) (Yong et al., 1998). Leukocytes from the peripheral blood may also contribute to the concentration of MMP-9 during CNS diseases (Bergman et al., 2002).

CNS expression of MMP-2 has been documented in humans without CNS inflammation or BBB disturbances as well as in healthy experimental animals (rats and rabbits); concentrations do not increase dramatically during pathological conditions (Paul et al., 1998; Azeh et al., 1998; Yushchenko et al., 2000). MMP-9 has not been found in the CSF of healthy animals or controls in other studies (Gijbels et al., 1992; Perides et al., 1998; Kolb et al., 1998), but a high correlation exists with the nucleated

cell count (Yushchenko et al., 2000). Granulocytes and macrophages are strong producers of MMP-9, whereas lymphocytes are weak producers, thus CSF microscopic analysis maybe predictive of the MMP-9 levels. Bacterial meningitis in people results in high levels of MMP-9 which may be due to the invasion of large numbers of granulocytes (Gijbels et al., 1992; Paul et al., 1998; Kieseier et al., 1999). Levels of MMP-9 are low to moderately increased in viral meningoencephalitis in the presence of high concentrations of lymphocytes (Yushchenko et al., 2000). If CSF is dominated by lymphocytes, the presence of detectable MMP-9 may be due to the presence of macrophages (Yushchenko et al., 2000).

The increase of MMP-9 activity in CSF of dogs may not help to make a diagnosis of a specific neurological disease, but it may be useful as a marker of the various neuroinflammatory diseases of dogs (Bergman et al., 2002). For instance, elevated MMP-9 activity in the CSF and serum has been reported in dogs with acute spinal cord trauma from intervertebral disc disease (IVDD) (Levine et al., 2006). This study showed that dogs with IVDD often expressed MMP-9 in the CSF when neurological dysfunction had been present for <24 h. Dogs with paraplegia due to IVDD, more frequently expressed MMP-9 than those with paraparesis and ataxia or no neurological deficits (Levine et al., 2006). Further investigations are required of CSF and serum MMP expression in dogs with IVDD as well as other neurological diseases to better assess the prognostic value of MMP-9 in the CSF and to evaluate the potential benefit of MMP-9 inhibitors (Levine et al., 2006).

Protein electrophoresis

Qualitative CSF protein analysis is performed to characterise the distribution of protein in the CSF, and often is very useful to compare the distribution with the ones found in serum for a better understanding of the pathological status of the patient. Several techniques are available, especially in human laboratories (agarose gel electrophoresis, immunoelectrophoresis, and isoelectric focusing) (Sorjonen et al., 1991; Reiber et al., 2003); however, high resolution agarose gel electrophoresis (HRE) produces sharper bands and definition of CSF protein fractions in people (Fishman, 1992), horses (Furr et al., 1997), and dogs (Sorjonen, 1987; Behr et al., 2006).

Abnormalities in CSF protein electrophoretic patterns have been reported to be useful in the identification of inflammatory, neoplastic, and degenerative disease (Sorjonen, 1987; Sorjonen et al., 1989). Dogs with canine distemper viral disease often have absolute elevation in CSF gamma globulins (probably due to intrathecal production) (Sorjonen et al., 1989), and dogs with granulomatous meningoencephalomyelitis may have an increase in beta and gamma globulins (Chrisman, 1992). CSF protein electrophoresis may be helpful to differentiate dogs with inflammatory disease that produce mild BBB impairment from those that have intrathecal production of gamma globulins (Sorjonen et al., 1989) However, in general, CSF electrophoresis cannot be confirmed as a valuable ancillary diagnostic tool for neurological disease (Behr et al., 2006).

Flow cytometry

Flow cytometric analysis of CSF is largely performed in human medicine to demonstrate the presence of lymphocyte clones in the diagnosis of lymphomas and lymphoproliferative disorders (Babusikova and Zeleznikova, 2004) (Subira et al., 2002). In veterinary medicine, few studies have reported the use of flow cytometry and immunophenotyping to identify mononuclear cells in the CSF of inflammatory conditions (Duque et al., 2002). Flow cytometric methods can provide information on the cellular phenotypes present in the CSF. Recently, two cases of CNS lymphoma were immunophenotyped as B- or T-cell lymphomas using CSF samples and flow cytometric techniques in place at one of

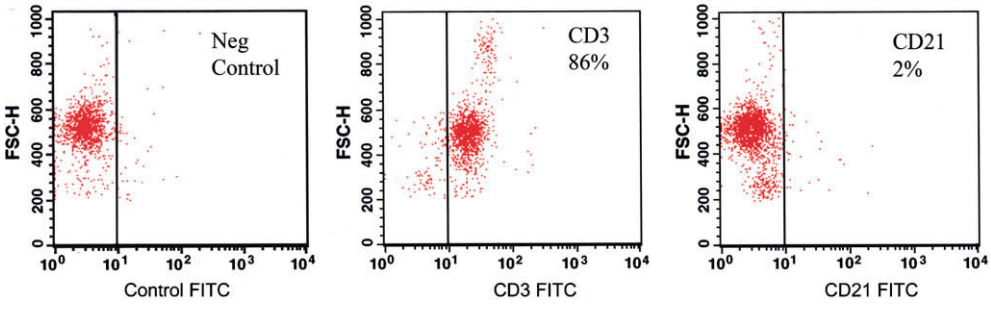

Fig. 3a. Flow cytometry plots of CSF from a 7-year-old, female, spayed, Golden Retriever presented to the College of Veterinary Medicine, Kansas State University with hind limb ataxia and weakness. CSF collected from CMC was colourless, hazy with a total protein concentration of 145 mg/dL and TNCC 3175 cell/μL. A lymphocytic pleocytosis was present on cytological examination. Flow cytometry of CSF cells labelled with fluorochrome-conjugated antibodies to specific cell markers (CD3 for T cells and CD21 for B cells) are listed on the *X*-axis. A vertical line is placed to the right of the cells labelled with the negative control antibodies and is used to identify populations of cells that expressed CD molecules and shifted to the right in fluorescent intensity (log scale). In this case, 86% of the cell population expressed CD3, and only 2% expressed CD21, consistent with a T-cell lymphoma (Courtesy Dr. Melinda Wilkerson, Manhattan, KS).

the author's institutes (Fig. 3). This technique allows one to identify the lineage of the neoplastic cells even when the distinction between monocytes and lymphocytes is difficult via microscopy. The use of this diagnostic modality is hindered by the practical issue that larger volumes (about 4–5 mL) of CSF than are usually available from dogs and cats are necessary unless the cell count is very high.

Microscopic evaluation

Cytological microscopic slide preparation

Because of the low numbers and extreme fragility of CSF cells, concentration of the cells is required for cytological evaluation. Several procedures are available for the morphological study of CSF cells. Most laboratories use a cytocentrifuge to prepare cells for evaluation, (Garma-Avina, 2004). A cytocentrifuge uses a slow speed centrifugation and acceleration (around 100–150 *g* based on the one we use, a Cytospin 2 [Shandon], for 4–6 min) to concentrate the cells from 200–400 μL (depending on the numbers of cells in the fluid) into a small circular area on a glass slide (Christopher et al., 1988). The cellular detail is excellent because the elements are gently spread out on the slide; however, there are some artifactual changes in the cells such as increased vacuolation and alteration in structure, particularly of monocytoid cells and macrophages.

Other techniques available including sedimentation and membrane filtration have already been described (Garma-Avina, 2004). An inexpensive in-house sedimentation chamber has been described to obtain cytological specimens of CSF. This device may be very useful when access to a laboratory is difficult, or when a cytocentrifuge is not available. The results obtained using this technique are consistent, and it also permits retrieval of the cell-free fluid for its use in chemical or immunological procedures (Garma-Avina, 2004).

Cytocentrifuge and sedimentation preparations are most commonly air-dried and stained with Romanowsky stains (May-Grünwald-Giemsa Wright-Giemsa or Diff-Quick). Special stains are indicated in some cases: Gram staining may be useful for confirmation and identification of bacteria (Cook and DeNicola, 1988; Evans, 1988). India ink or new methylene blue could be helpful in identification of fungal infections, especially cryptococcosis (Cook and DeNicola, 1988). Periodic acid-Schiff stain may be useful to demonstrate positive intracellular material in animals with storage diseases such as globoid cell leukodystrophy or mucopolysaccharidosis (Roszel et al., 1972). Luxol fast blue can be helpful to confirm the presence of myelin in CSF samples (Mesher et al., 1996).

Cytological microscopic examination

Cytological CSF examination is essential even if the TNCC is within normal limits because it is still possible to have abnormalities in cell type or structure (Evans, 1988). The absence of any abnormality in CSF does not rule out the possibility of neurological disease. In all cases, cytocentrifugation is recommended to concentrate cells with minimal cell loss.

Blood contamination

CSF does not normally contain erythrocytes (Cook and DeNicola, 1988; Chrisman, 1992). The presence of erythrocytes in a sample is most commonly iatrogenic in origin (contamination during collection), but otherwise suggests a pathological subarachnoid haemorrhage. Numerous factors and formulas have been used to correct protein concentration and TNCC for the effect of blood contamination in CSF, but most are considered inaccurate (Sweeney and Russell, 2000). In feline CSF, a red blood cell count >30 cells/μL can have effect on the total and differential cell count (Rand et al., 1990). However, in a recent canine study, the RBC count was not significantly

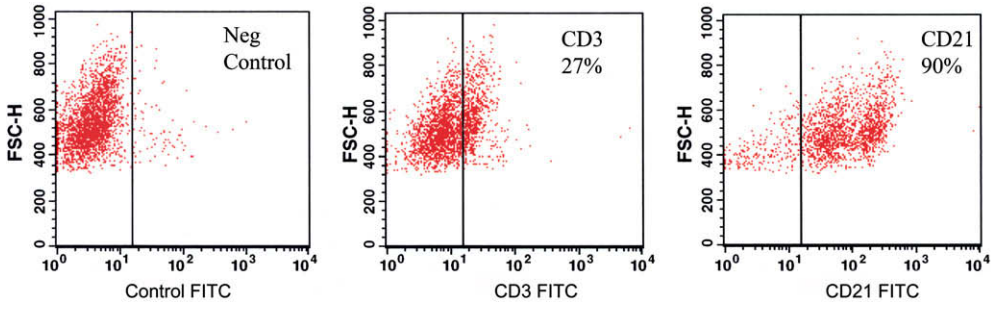

Fig. 3b. Flow cytometry plots of CSF from a 10-year-old, female, spayed, Beagle presented at Kansas State University, College of Veterinary Medicine VMTH with cervical pain. CSF collected from CMC was opaque and cloudy, with a total protein concentration of 117 mg/dL and TNCC 9387 cell/μL. A lymphocytic pleocytosis was present (>95% lymphoblasts) on cytological examination. Flow cytometry of cells obtained from CSF were labelled with fluorochrome-conjugated antibodies to specific cell markers (CD3 for T cells and CD21 for B cells) as listed on the *X*-axis. A vertical line is placed to the right of the cells labelled with the negative control antibodies and is used to identify populations of cells that expressed CD molecules and shifted to the right in fluorescent intensity (log scale). In this case, 90% of the cell population expressed CD21, and 27% expressed CD3, consistent with a B-cell lymphoma (Courtesy of Dr. Melinda Wilkerson and Dr. Mehrdad Ameri, Manhattan, KS).

associated with TNCC or protein concentrations in CSF from clinically normal dogs or those with evidence of neurological disease, even if the sample contained up to 13,200 RBC/μL (Hurtt and Smith, 1997).

Normal nucleated cells in the CSF

Mononuclear cells predominate in the CSF of healthy dogs and cats and consist of normal looking small lymphocytes, which are very similar to the same cells seen in the blood, and larger cells classified as monocytoid cells (Cook and DeNicola, 1988; Rand et al., 1990; Chrisman, 1992; Parent and Rand, 1994). Low numbers (up to 10% of TNCC) (Chrisman, 1992) of mature, non-degenerate neutrophils are occasionally seen in normal CSF and probably result from systemic blood contamination at the time of sampling. Some authors, however, have suggested that the presence of neutrophils or eosinophils in the CSF is indicative of an abnormality (Christopher et al., 1988). In cats, on the other hand, up to 8% of nucleated cells can be neutrophils in non-blood contaminated CSF (Rand et al., 1990). Occasionally, ependymal cells, choroid plexus cells, meningeal lining cells, or mitotic figures may be seen in samples of normal individuals (Cook and DeNicola, 1988; Rand et al., 1990; Chrisman, 1992; Parent and Rand, 1994). Published reference intervals for canine CSF TNCC are reported to be <5 cells/μL (Cook and DeNicola, 1988; Oliver and Lorenz, 1997).

Lymphocytes

Small lymphocytes (Fig. 6) are the most common cell type in normal CSF of dogs; they may also be called small mononuclear cells. They are 9–15 μm in diameter, with dark, compact, round to slightly oval or indented nuclei and usually have a narrow border of pale-blue cytoplasm. A nucleolus is occasionally visible. Medium and large lymphocytes (measured based on nuclear size) are not usually a constituent of normal CSF (Cook and DeNicola, 1988; Chrisman, 1992). Their nuclei are often larger with a less dense and more scattered chromatin pattern than the small lymphocytes. The cytoplasm is much more abundant and may contain a few dense azurophilic granules.

The presence of medium and large lymphocytes suggest the possibility of a pathological process, even if the TNCC is not increased, especially if there is a local antigenic stimulation within the subarachnoid space (Grevel and Machus, 1992). Occasionally plasma cells may be observed with active or resolving infectious diseases, neoplastic processes, and potential immune-mediated diseases (Cook and DeNicola, 1988). The origin of lymphocytic cells in the CSF is not well known; the majority may be derived from leptomeningeal stem cells and migrate from the blood to the CSF (Sayk, 1962). It is also possible that the lymphocytes return to the blood, accounting for a regular circulation and exchange (Herndon and Brumback, 1989).

Monocytes

Monocytes (Fig. 7a) are less common than lymphocytes in the normal CSF of dogs, whereas in cats these cells seem to be the predominant components of normal CSF (Cook and DeNicola, 1988; Rand et al., 1990; Parent and Rand, 1994). Monocytoid cells compose 69–100% of the TNCC, lymphocytes 0–27%, neutrophils 0–9%, macrophages 0–3%, and eosinophils 0 to <1% of the CSF in healthy cats. Those cells may also be called large mononuclear or monocytoid cells.

The cells are large (12–15 μm) in diameter and have oval, sometimes kidney-shaped, or slightly lobulated nuclei that stain less intensely than the nuclei of small lymphocytes (Cook and DeNicola, 1988; Chrisman, 1992). They have moderate to abundant light-blue cytoplasm, which is foamy or finely vacuolated. In pathological conditions, monocytes are transformed into macrophages that contain varying amount of phagocytised material, such as lipid droplets, erythrocytes, microorganisms, and cell debris in various stages of digestion. Activated monocytoid cells and macrophages may appear in clusters mimicking epithelial cells (Chrisman, 1992).

Uncommon, incidental components and other findings

Leptomeningeal lining cells, choroid plexus cells, and ependymal cells can occasionally be found in normal CSF. They are more commonly observed if aspiration has been used to collect the fluid (Cook and DeNicola, 1988). The leptomeningeal lining cells often appear in small clusters and consist of mononuclear cells with moderate to abundant amounts of pale basophilic cytoplasm, round to oval eccentric nuclei and indistinct cytoplasm margins.

Choroid plexus cells and ependymal cells are difficult to distinguish; both elements are very fragile, small, columnar to cuboidal and of uniform size and morphology (DeMay, 1996) with round, often pyknotic-appearing nuclei and a wide border of pink or blue-grey cytoplasm. These cells, which usually appear in groups, derive from the epithelium of the cerebral ventricles or from the choroid plexus and can appear frequently in the CSF of small children associated with hydrocephalus (DeMay, 1996). These cells are rarely seen in veterinary medicine, but it is important not to interpret them as neoplastic cells when identified.

It is possible to find elements that originate from the path of the spinal needle. These often include cartilage cells and epithelial cells. Bone marrow elements may be found as contaminants in canine CSF associated with bone marrow aspiration during lumbar cistern collections of CSF (Christopher, 1992).

Intracellular myelin (within macrophage cytoplasm) has been found in the CSF from a dog with myelomalacia (Mesher et al., 1996). Macrophages on the cytocentrifuge preparation stained positively with Luxol fast blue (LFB), which specifically stains myelin. The myelin-like structures described in other cases reported may be referred

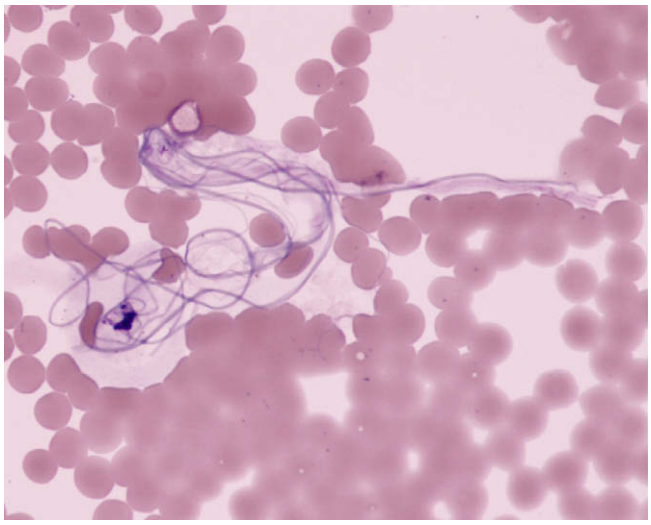

Fig. 4. Myelin figures in CSF from a dog. Extracellular myelin fragments are the grey-blue ribbon structures which are phospholipids from damaged axons (Wright-Giemsa; ×100) (Courtesy of Dr. Steve Stockham, Manhattan, KS).

to as either myelin 'figures' or myelin 'fragments', which appear to be very similar on light microscopy (Bauer et al., 2006). A consistent difference between these two structures is that myelin 'figures' originate from a non-specific lipid source; they are laminated lipids arranged in coils or stacks derived from cell membrane phospholipids and cellular organelles as a result of cell death or injury. Myelin figures may be found extracellularly (Fig. 4) or as engulfed material within macrophages (Ghadially, 1988).

Myelin 'fragments' specifically denote a collection of myelin found only extracellularly, and are thus are more indicative of demyelination (Ghadially, 1988). However, it is important to remember that during demyelination, the lipid originated from myelin undergoes a denaturation change including the formation of free neutral fats which will not stain with LFB (Jones and Hunt, 1983). The only reliable way to differentiate between myelin figures and myelin fragments is to examine the laminated structure on electronic microscopy specifically for the appearance, periodicity and location of the myelin bands relative to the cells (Fallin et al., 1996).

Other rare or occasional findings in CSF samples include neural elements such as neuroglial cells, ventricle associated cells (ependymal and choroidal plexus cells), and meningeal lining cells (Chrisman, 1992). Nervous tissue has also been rarely identified in a few cases after routine CSF cytological analysis, and reported as contamination due to accidental puncture of the spinal cord during cerebello-medullary cisternal collection (Fallin et al., 1996).

Interpretation of cytological abnormalities

TNCCs may be within normal reference intervals but the cytocentrifuged CSF may demonstrate increased percentages of cells such as neutrophils and eosinophils in a variety of neurological disorders (Freeman and Raskin, 2001). If blood contamination is not the cause, increased neutrophil percentages of >10–20% (Chrisman, 1992; Meinkoth and Crystal, 1999) and eosinophil percentages of >1% should be considered abnormal (Meinkoth and Crystal, 1999). An increased percentage of neutrophils without increases in TNCC may indicate a mild or early CNS inflammation, a lesion that does not contact the meninges or ependymal cells or previous use of drugs such as glucocorticoids and antibiotics, which may reduce the presence of an inflammatory response (Meinkoth and Crystal, 1999). An increase in the neutrophil percentage with normal TNCC has been reported in acute IVDD, vertebral fractures (Thomson et al., 1989), cerebrovascular accidents, (infarct/haemorrhage) and in fibrocartilagenous thromboembolism in dogs (Bailey and Vernau, 1997).

Eosinophils are considered an abnormal finding in canine and feline CSF. An increased eosinophil percentage without increased TNCC has been described concurrently with either parasite migration (e.g., *Parelaphostrongylus tenuis* in llamas) or protozoal disease (*Neospora* spp. in dogs) (Chrisman, 1992).

An increase in TNCC in the CSF is referred to as pleocytosis, which may be considered *mild* (<25 cells/μL), *moderate* (26–100 cells/μL), or *marked* (>100 cells/μL) and is further defined by the predominant cell type in the sample as neutrophilic, eosinophilic, mononuclear, or mixed (Freeman and Raskin, 2001). The degree of pleocytosis is due to several factors, including the cause, the severity and the location of the lesion with respect to the subarachnoid space or ventricular system (Cook and DeNicola, 1988). As mentioned above, it is important to remember that normal CSF results do not exclude the presence of disease (Kjeldsberg and Knight, 1986; Fishman, 1992), especially with deep parenchymal lesions which do not communicate with the leptomeninges, the subarachnoid space or the ependymal surfaces (Cook and DeNicola, 1988). Cytological changes must always be interpreted in light of the patient's history and neurological findings. However, abnormal CSF findings always indicate the presence of a pathological abnormality if blood contamination has been ruled out.

Neutrophilic pleocytosis

Neutrophilic pleocytosis (Fig. 5) has been described in a wide range of acute inflammatory diseases that include trauma, haemorrhagic diseases, post myelographic reactions as well as bacterial and fungal infections, immune-mediated disease, and neoplasia (Coles, 1986; Cook and DeNicola, 1988; Chrisman, 1992; Meinkoth and Crystal, 1999). In dogs, steroid-responsive meningitis–arteritis (SRMA) and necrotising vasculitis is usually associated with a neutrophilic response (Meric, 1988). The neutrophils are well-preserved, generally non-degenerate or hypersegmented, and CSF cultures are negative for bacteria (Mein-

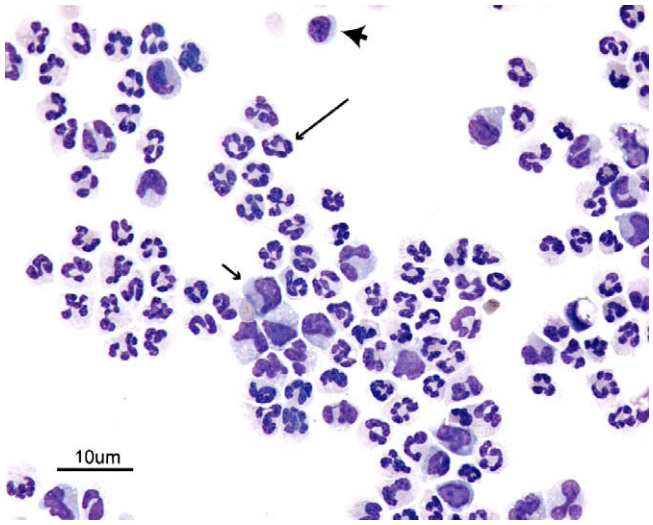

Fig. 5. Neutrophilic pleocytosis in the CSF of a 1-year-old mixed breed dog with steroid responsive meningo-arteritis (SRMA). Many non-degenerate neutrophils (long arrow), a few monocytes (short arrow) and scattered small lymphocytes (arrowhead) are present (Wright-Giemsa).

koth and Crystal, 1999). SRMA may be associated with neutrophilic pleocytosis >500 cells/μL, with a neutrophilic percentage of between 75% and 100% (Chrisman, 1992).

Recent retrospectives of dogs with bacterial meningitis showed that 98% had neutrophilic pleocytosis (Tunkel and Scheld, 1993). However, when a bacterial or fungal meningitis has been treated with antibiotics for a week or more, mononuclear cells may be found in elevated percentages replacing neutrophils as the predominant cell (Kornegay et al., 1978; Bullmore and Sevedge, 1978; Dow et al., 1988). Cytological evaluation of the CSF for the presence of intracellular rods or cocci is essential in cases of suspect bacterial CNS disease.

Feline infectious peritonitis (FIP) is another common cause of marked neutrophilic pleocytosis (>50% neutrophils) with elevated TNCC (>100 μL^{-1}) in cats and is often associated with increased CSF total protein (usually >200 mg/dL) (Foley et al., 1998). Necrosis associated with inflammation induced by neoplasia within the brain and severe seizure activity may also cause a mild neutrophilic pleocytosis or an increased neutrophil percentage with normal TNCC (Bailey and Higgins, 1986).

CNS neoplasia may induce tumour necrosis, thus a reactive neutrophilic inflammation may be found in the CSF (Vandevelde and Spano, 1977). Several studies (Bailey and Higgins, 1986; Grevel et al., 1992) have described abnormal CSF in cases of intracranial meningiomas in dogs, and that the majority of the samples had a neutrophilic pleocytosis (neutrophils >25%); however, a recent study showed that 16% (12/77) of dogs with intracranial meningiomas had normal CSF. Pleocytosis was detected in only 27% (20/77) of the dogs, and pleocytosis with a predominance of neutrophils was found in only 19% (15/77) of the dogs (Dickinson et al., 2006). The location of the tumour plays a significant role in the CSF changes and neutrophilic pleocytosis may not be detected in CSF samples from dogs with meningiomas located within the middle or rostral portion of the cranial fossae (Dickinson et al., 2006). Practically, most intracranial neoplasia is detected with advanced imaging and so CSF analysis may not be performed due to the concerns of subsequent parenchymal shift or due to its inability to add further information.

Mononuclear pleocytosis

Mononuclear pleocytosis occurs with an increase in the percentage or concentration of small and mature lymphocytes (>70%) (Fig. 6) and/or an increase in the number of monocytoid/macrophage cells (Figs. 7a and 7b). The presence of reactive lymphocytes with normal TNCC may be a sign of CNS disease (Coles, 1986; Christopher et al., 1988).

An alteration in both the number and structure of lymphocytes occurs in a variety of diseases such as CNS viral infection in dogs (Vandevelde and Spano, 1977) and cats (Rand et al., 1994a). Canine distemper virus (CDV) infection is typically associated with a mild to moderate lymphocytic pleocytosis. The CSF in CDV cases may show an increase in macrophages, and occasionally intracytoplasmic CDV inclusion bodies are recognised (Abate et al., 1998). Granulomatous meningo-encephalomyelitis (GME) is another frequent cause of mononuclear pleocytosis, even though it may have an extremely variable spectrum of cytological findings. The CSF in GME may have a mild to moderate lymphocytic, neutrophilic or mixed cells pleocytosis (Chrisman, 1992; Munana and Luttgen, 1998).

In Pugs, Yorkshire terriers and Maltese terriers, a moderate to marked lymphocytic pleocytosis with >80% lymphocytes is consistent with a necrotising meningo- or leuko-encephalitis, although a mixed cell pleocytosis may

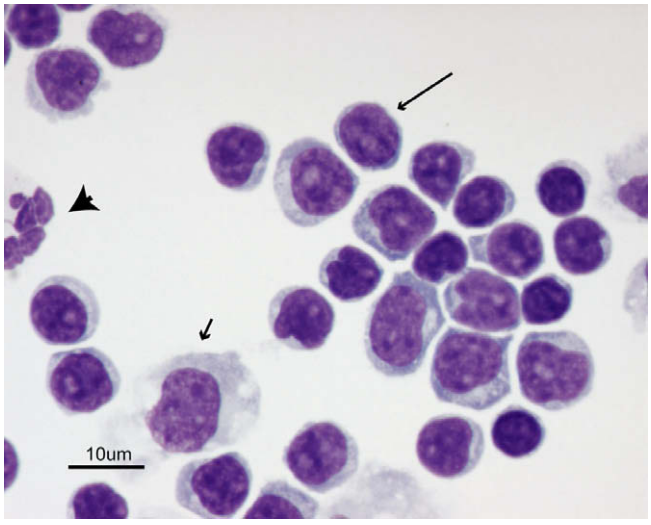

Fig. 6. Mononuclear pleocytosis (lymphocytic) in the CSF of an 8-month-old Pug with necrotising meningoencephalitis. Many small lymphocytes (long arrow), few monocytes (short arrow) and rare neutrophils (arrowhead) are present (Wright-Giemsa).

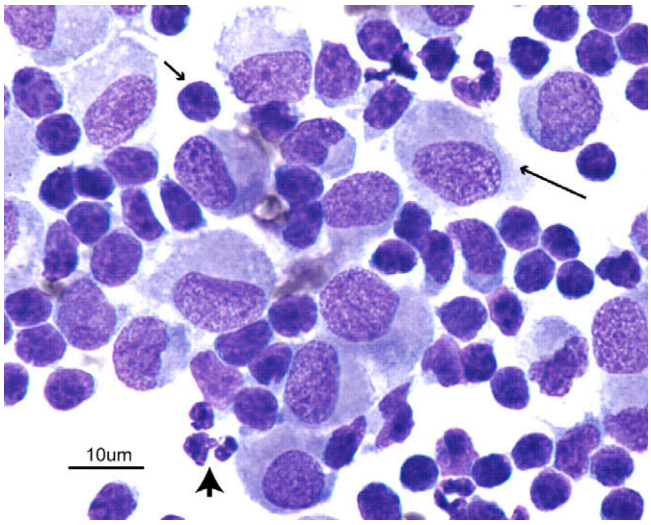

Fig. 7a. Mononuclear pleocytosis (monocytic/macrophagic) in the CSF of a 4-year-old Weimaraner with granulomatous meningoencephalitis (GME). Many monocytoid cells (long arrow), small lymphocytes (short arrow), and rare neutrophils are present (Wright-Giemsa).

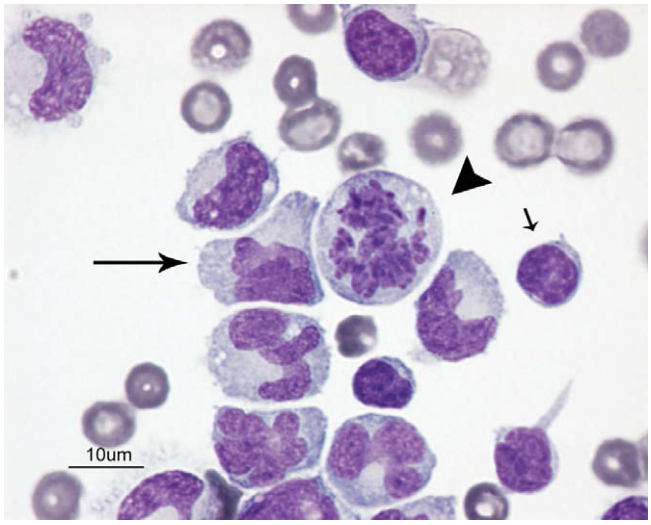

Fig. 7b. Mononuclear pleocytosis (monocyto/macrophagic) in the CSF of a 4-year-old Weimaraner with GME. Many monocytoid cells (long arrow), few small lymphocytes (short arrow), and occasional mitotic figures (arrowhead) are present. (Wright-Giemsa).

be seen (Freeman and Raskin, 2001). Large numbers of granular lymphocytes have also been described in a dog with necrotising meningo- or leuko-encephalitis (Garma-Avina and Tyler, 1999).

Marked lymphoid pleocytosis has been seen in some animals with a CNS lymphoma (Long et al., 2001). When these cells represent immature lymphocytes (lymphoblasts), lymphoma is easily differentiated cytologically (Freeman and Raskin, 2001; Long et al., 2001). However, well-differentiated lymphoid malignancies may not be easily differentiated from a lymphocytic pleocytosis (Freeman and Raskin, 2001). Other diseases in which lymphocytic pleocytosis is seen include ehrlichiosis, toxoplasmosis, neosporosis, and in some cases of bacterial meningitis following antibiotic treatment (Fishman, 1992; Rand et al., 1994b; Thomas, 1998; Radaelli and Platt, 2002).

Mixed cell pleocytosis

Mixed cell pleocytosis (Fig. 8) describes a mixed cell population of lymphocytes, monocytoid/macrophages, neutrophils and a few to rare eosinophils and plasma cells. The most common disease demonstrating CSF mixed cell pleocytosis is canine GME, but all the other possible differentials cannot be ruled out. The chronic phase of SRMA should also be considered as well as fungal infections such as cryptococcosis (Fig. 9), histoplasmosis, blastomycosis and aspergillosis, ehrlichiosis, toxoplasmosis, neosporosis, and protothecosis (Chrisman, 1992; Meadows et al., 1992).

Mild to moderate mixed cell pleocytosis may result from necrosis or inflammation secondary to a variety of diseases such as disc disease, haemorrhagic myelomalacia, ischaemia, or infarction (Chrisman, 1992; Freeman and Raskin, 2001). A mixed cell pleocytosis with mononuclear atypia has been reported in a dog with primary CNS malignant histiocytosis (Zimmerman et al., 2006).

Eosinophilic pleocytosis

Eosinophils are not present in normal CSF unless blood contamination has occurred. Peripheral blood eosinophilia is not always associated with CSF eosinophilic pleocytosis, and when present there is no association between the magnitude of the peripheral eosinophilia and the severity of the CSF eosinophilia (Smith-Maxie et al., 1989). Eosinophilic pleocytosis con-

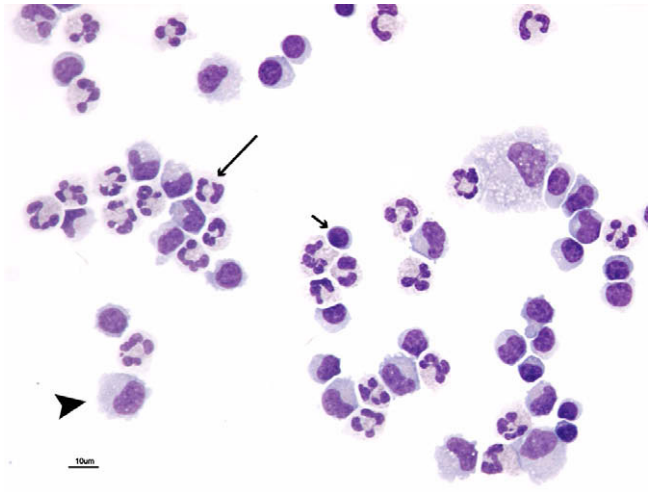

Fig. 8. Mixed cell pleocytosis in the CSF of a 6-year-old English Springer spaniel with GME. A mixture of non-degenerate neutrophils (long arrow), monocytes/macrophages (short arrow) and small lymphocytes (short arrow) are present in roughly equal numbers (Wright-Giemsa).

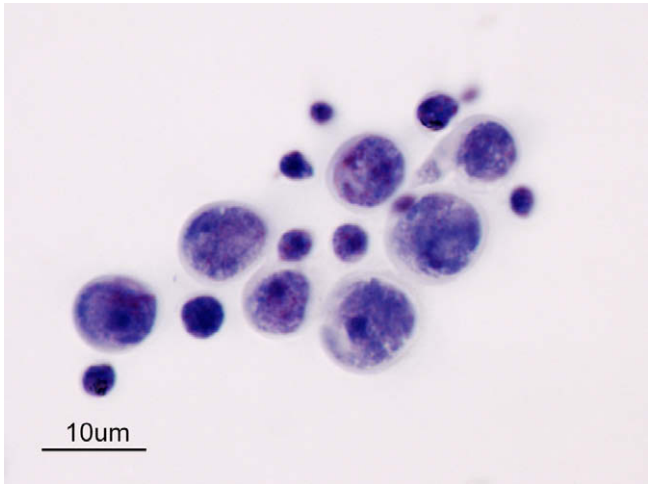

Fig. 9. Cryptococcosis in the CSF of a 1-year-old Miniature Schnauzer. Many extracellular yeasts consistent with *Cryptococcus neoformans* are present (Wright-Giemsa).

sisting predominantly of eosinophils is infrequent (Chrisman, 1992). Non-specific acute inflammatory responses may increase the number of the eosinophils in the CSF. This condition has been described as steroid responsive eosinophilic meningoencephalitis, and has been reported in dogs (Smith-Maxie et al., 1989) and cats (Schultze et al., 1986). Some breeds of dogs, such as Golden retrievers and Rottweilers may be predisposed (Smith-Maxie et al., 1989; Bennett et al., 1997).

Eosinophilic pleocytosis has also be seen in protozoal infections such as toxoplasmosis, neosporosis, and fungal diseases such as cryptococcosis (Cook and DeNicola, 1988; Chrisman, 1992). Eosinophilic pleocytosis has also been reported in animals with aberrant parasitic migrations as is found, for example, with *Parelaphostrongylus tenuis* in llamas (Fig. 10), protothecosis, and very rarely in CDV infection, and rabies (Chrisman, 1992).

Conclusions

CSF analysis forms a vital part of the panel of diagnostic tests required for the assessment of neurological cases. It infrequently provides a definitive diagnosis, but if the analysis is performed thoroughly it may assist in the diagnosis of underlying diseases in conjunction with advanced imaging and systemic health assessments to better define a more specific differential diagnosis (see Figs. 11 and 12).

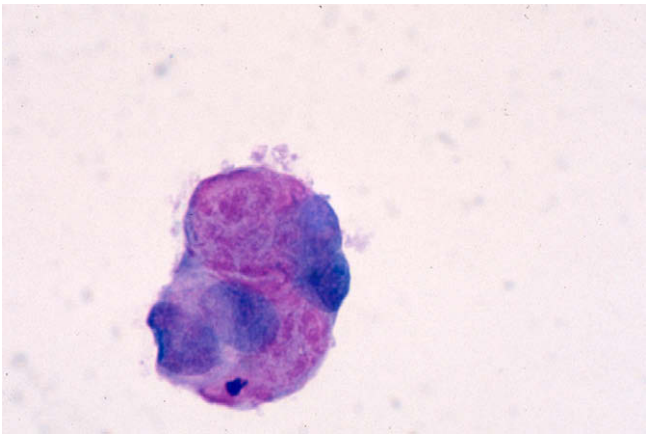

Fig. 11. Meningioma in the CSF of a 13-year-old mixed breed dog. Nests of large cohesive cells of a meningoepitheliomatous appearance are present. Cells contain eosinophilic secretory material (Wright-Giemsa; ×100) (Courtesy of Dr. Steve Stockham, Manhattan, KS).

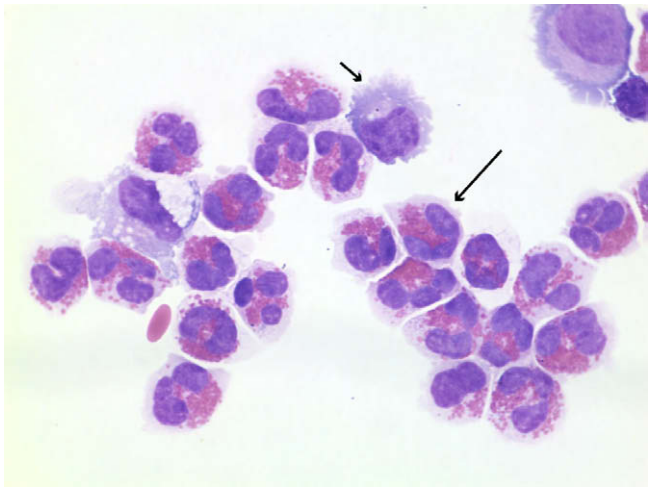

Fig. 10. Eosinophilic pleocytosis in the CSF from a llama. Many eosinophils (long arrow) and occasional macrophages (short arrow) are present. The llama was infected by the meningeal worm *Parelaphostrongylus tenuis* (Wright-Giemsa; ×100) (Courtesy of Dr. Karen Young, Madison, WI).

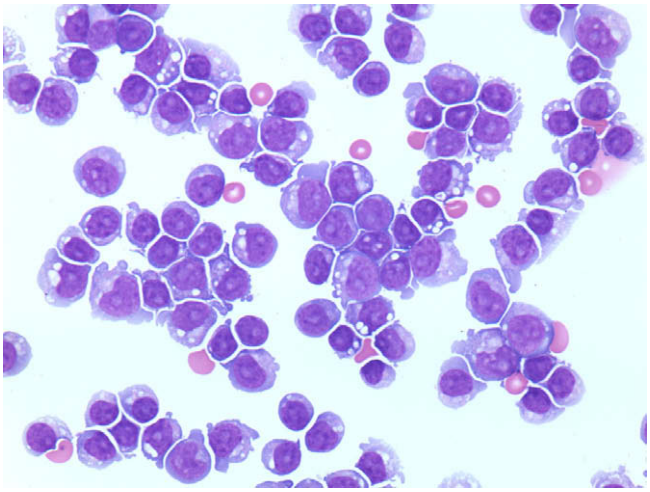

Fig. 12. Lymphoma in the CSF of a 9-year-old Labrador Retriever. Medium to large lymphocytes with immature chromatin, prominent nucleoli and basophilic, often vacuolated, cytoplasm are present (Wright-Giemsa; ×100) (Courtesy of Dr. Karen Young, Madison, WI).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tvjl.2007.11.024.

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