Research paper

Diagnostic value of cerebrospinal fluid analysis in a population of dogs with suspected idiopathic Epilepsy

Ana M. Coelho a, Thomas W. Maddox b, Daniel Sanchez-Masian b, Rita Gonçalves b,*

a Dick White Referrals, London Road, Six Mile Bottom, Cambridge CB8 0UH, UK
b Department of Small Animal Clinical Science, Institute of Veterinary Science, University of Liverpool, Neston CH64 7TE, UK

* Corresponding author. Tel.: +44 151 7956100.
E-mail address: r.goncalves@liv.ac.uk (R. Gonçalves).

Keywords: Seizures; Epilepsy; Disease Investigation; MRI; Dog

Any grant or other financial support: NA
Abstract

Cerebrospinal fluid (CSF) analysis is commonly used in the diagnostic investigation of seizure disorders in order to exclude possible inflammatory underlying aetiology.

The medical records were searched for dogs presenting with epileptic seizures (ES) that had normal interictal neurological examination, normal complete blood count and biochemistry analysis, unremarkable magnetic resonance imaging of the brain and had CSF analysis performed as part of the diagnostic investigation.

A total of 200 dogs met the inclusion criteria. The CSF was abnormal in 30 dogs with a median total nucleated cell count of 2 cells/µl (IQR 1.5-6) and median protein concentration of 0.37g/l (IQR 0.31-0.41). Pleocytosis was recorded in 14/30 dogs and the CSF protein was increased in 22/30. There was no correlation between abnormal CSF and the type or number of seizures or the time interval between the last seizure and CSF collection. A significant correlation was found between the number of red blood cells on CSF and having an abnormal CSF. The prevalence of having a diagnosis other than suspected idiopathic epilepsy (IE) was 0.5% (1/200).

These results suggest that performing CSF analysis in dogs with recurrent ES that have normal interictal neurological examination and unremarkable MRI has a low diagnostic value.
Introduction

Epileptic seizures are the most common chronic neurological condition in dogs.\(^1\) Epilepsy is a complex disease of the brain characterised by an enduring predisposition to generate epileptic seizures and is practically defined as having at least two unprovoked epileptic seizures >24 h apart. According to its aetiology, it can be classified into idiopathic epilepsy (IE), whereby an underlying cause for the epileptic seizures (ES) cannot be found or structural epilepsy, in which there is an underlying intracranial pathology.\(^2\) Reactive seizures occur in response to a transient disturbance in function (metabolic or toxic in nature), which is reversible when the cause or disturbance is rectified.\(^2\) The prevalence of idiopathic epilepsy in the general canine population of the United Kingdom (UK) has been reported as 0.62\%.\(^3\)

Diagnosing IE is achieved by excluding other possible causes of ES. The minimum criteria for a Tier I confidence level diagnosis of IE includes confirmation of two or more unprovoked ES 24 h apart, an onset of the episodes between 6 months of age and 6 years, an interictal period exempt of neurological deficits and non-clinically significant abnormalities on minimum database blood tests and urinalysis.\(^4\) A Tier II confidence level for the diagnosis of IE is attributed when structural epilepsy has also been excluded through magnetic resonance imaging (MRI) of the brain, CSF analysis and unremarkable fasting and post-prandial bile acid stimulation test.\(^4\) Financial considerations and owner preference to avoid potentially more invasive procedures (such as general anaesthesia and CSF collection) can dictate investigation, and in such cases, the ability to suggest a diagnosis prior to further investigations is valuable.

Previous studies have shown that the neurological examination alone is a good predictor for an abnormal MRI in epileptic dogs.\(^5\) \(^6\) \(^7\) The studies have suggested that the sensitivity for finding abnormalities on MRI in dogs without interictal neurological deficits is low and that this test may not always be essential in dogs less than 6 y old.\(^6\)
The value of performing CSF collection and analysis in dogs with suspected IE, after reactive seizures and structural abnormalities on MRI have been excluded, has not yet been determined. The aim of this study was therefore to determine the value of performing CSF analysis in a population of dogs with normal neurological examination in the interictal period, unremarkable haematology and biochemistry and MRI of the brain.

Materials and methods

The medical records from the Neurology and Neurosurgery service of the Small Animal Teaching Hospital (SATH) of the University of Liverpool between 2010 and 2015 were reviewed retrospectively to identify dogs presented for investigation of ES. The study protocol was approved by the University of Liverpool’s Veterinary Research Ethics Committee (VREC263).

Inclusion criteria were that the patients had (1) presented for investigation of ES, (2) unremarkable interictal neurological examination performed by either a board-certified, board eligible neurologists or residents, (3) normal complete blood count (CBC), normal biochemistry profile (or clinically non-significant biochemistry alterations), (4) normal MRI of the brain and (5) cerebrospinal fluid (CSF) analysis performed. Data related to signalment, age at ES onset, ES semiology (focal or generalised), presentation of cluster seizures (defined as 2 or more ES within a 24h period) or status epilepticus (ES which shows no signs of arresting after a duration encompassing the great majority of ES of that type, defined clinically as greater than 5 minutes), and number of ES episodes prior to investigation were documented. The time interval between the last ES and collection of CSF was also recorded. For statistical analysis, the dogs were divided into three groups according to a previous publication: length of time between their last ES and the CSF collection up to 48 hours (group 1); between 3 and 7 days (group 2) and over 7 days (group 3).
Diagnostic investigation in all cases included a CBC and serum biochemistry profile (sodium, potassium, chloride, calcium, phosphate, alanine aminotransferase, alkaline phosphatase, total bilirubin, urea, creatinine, total protein, albumin, glucose, cholesterol and triglycerides). A bile acid stimulation test and/or fasting ammonia concentration and abdominal ultrasonography were performed when hepatic encephalopathy was clinically suspected. *Neospora caninum* and *Toxoplasma gondii* antibody titres were tested in some cases. Dogs were excluded from the study if corticosteroids had been administered in the 7 days prior to presentation.

Magnetic resonance imaging was performed in all cases under general anaesthesia using a 1T (Siemens Magnetom, Erlangen, Germany) scanner. As a minimum, the following sequences were used: T2-weighted images (T2W) (in transverse, sagittal and dorsal planes), fluid attenuation inversion recovery (FLAIR) and pre and post-contrast (intravenous injection of 0.1mmol/kg of gadopentetate dimeglubine) T1-weighted images (T1W) in the transverse plane. A board-certified neurologist (RG) and a board-certified radiologist (TWM) examined all images and dogs with imaging abnormalities were excluded from the study.

The CSF collection was performed under the same general anaesthesia from either the cerebellomedullary cistern (CMC) or lumbar subarachnoid space (LSS) after advanced imaging; samples were collected into a plain plastic and EDTA tubes. The analysis was performed within one hour of collection and comprised a red blood cell (RBC) count and total nucleated cell count (TNCC), protein concentration measurement and a cytological examination with a differential cell count. The CSF samples with blood contamination of >5000RBC/μl were excluded from the study.

Results of CSF analysis were considered abnormal when the protein concentration was >0.30g/l (CMC) or >0.45g/l (LSS) or the TNCC was >5cells/μl. In the cases where blood contamination was present, formulae for CSF protein concentration and TNCC correction
previously reported were used to investigate the possible impact of the blood contamination on CSF analysis: the TNCC was increased by 1 nucleated cell/μl for every 500 RBC/μl and the protein concentration was increased by 0.01g/L for every 1,000 RBC/μl of CSF. The CBC results were also recorded in order to evaluate the influence of the CBC RBC and white blood cell (WBC) counts on the CSF results. The referring veterinarians of the patients that presented abnormal CSF results were contacted via telephone call for a progress update on the long-term outcome of these cases.

The sample size calculations indicated a total of 195-245 dogs would be required to estimate the prevalence with a precision of 5% and 95% confidence. All statistical analyses were performed with Minitab 14 (Minitab Inc. State College, Pennsylvania, USA) and R (“The R Project”, https://www.r-project.org). Descriptive statistics were computed for variables where appropriate; continuous data were summarised as median values with interquartile ranges (IQR), and categorical data were expressed as frequencies with 95% confidence intervals (95% CI). For categorical variables with a large number of categories and/or categories comprising only small numbers, groups were pooled into appropriate larger classes. For continuous variables normality of distribution was assessed graphically and using the Kolmogorov-Smirnov test. Associations between having an abnormal CSF analysis and categorical variables were assessed using Pearson’s Chi square or Fisher’s exact test, and associations with continuous variables were assessed using the Mann-Whitney U-test. The relationship between selected continuous variables was evaluated using Spearman’s rank correlation coefficient, in order to assess if increases in CSF blood contamination were associated with increases or decreases in CSF parameters or CBC parameters. For all analyses \( P<0.05 \) was considered significant.

**Results**
A total of 200 dogs with a history of ES and normal interictal neurological examination were included.

The CBC was normal in all cases. Biochemical abnormalities were found in 6 dogs and were mainly related to mild increment in liver enzymes (particularly serum alkaline phosphatase); 3/6 cases were receiving phenobarbitone. Serology antibody titres for *Toxoplasma gondii* and *Neospora caninum* were performed in 23/200 (11.5%) cases and were negative in all cases.

The majority of dogs were male (62.5%). The most prevalent breeds were: crossbreed (14.5%), Labrador retriever (8.5%), Border collie (8.5%) and Staffordshire bull terrier (5.5%). Median age at ES onset was 43.5 months (IQR 25-154) with 22 dogs (11%) <1y old, 130 dogs (65%) between 1y-6y and 48 dogs (24%) >6y old. Sixty-seven dogs (33.5%) presented focal ES and 133 dogs (66.5%) generalised ES according to the owners’ description. Cluster seizures were reported in 57 dogs (28.5%) and only 2 dogs (1%) had a previous episode of status epilepticus. Of the total population, 90 (45%) dogs suffered from ≤ 5 ES episodes prior to investigations and 110 (55%) of dogs had > 5 ES episodes recorded. The time interval between CSF collection and last ES episode was <2 days in 45 dogs (22.5%); between 3-7 days in 55 dogs (27.5%) and >7 days in 100 cases (50%).

The CSF samples were collected via CMC in 196 cases (98%) and LSS in 4 cases (2%). The median CSF RBC count was 1.5 (IQR 1.5-40) cells/µl, median TNCC was 1.5 cells/µl (IQR 1.5-2) and median protein concentration was 0.2 g/l (IQR 0.17- 0.26).

The CSF analysis was abnormal in 30 dogs (15%, 95% CI 0.11 – 0.21). For the abnormal cases, the median CSF RBC count was 40 cells/µl (IQR 2-360), median TNCC was 2 cells/µl (IQR 1.5-6) and median protein concentration was 0.37g/l (IQR 0.31-0.41). Of these, 1 dog had a history of status epilepticus and 6 dogs had cluster seizures reported. In 14/30 cases pleocytosis was identified with a median TNCC of 6 cells/µl (IQR 6-8). The samples were
collected from CMC in 13 dogs and in 1 dog from the LSS. The differential cell count of patients with pleocytosis revealed mononuclear predominance in all cases.

The CSF protein was increased in 22/30 dogs of which 21 had been sampled from the CMC and 1 from the LSS. The median value for abnormal CSF protein concentration collected from the CMC was 0.4g/l (IQR 0.36-0.42) and 0.46g/l on the case collected from the LSS. Both pleocytosis and raised CSF protein concentration were present in 6/30 cases and albuminocytological dissociation was present in 16/30 dogs.

The majority of dogs (16/30) with abnormal CSF had a time interval between the last ES and CSF analysis >7 days and 8/30 had CSF collected <2 days since the last ES. Twelve dogs with abnormal CSF analysis had suffered more than 5 ES prior to examination.

Associations between having an abnormal CSF and multiple independent variables were assessed (Table 1). The only significant correlation found was between the number of RBCs on CSF and having an abnormal CSF result (Mann-Whitney Test p<0.000). In only one of these cases the CSF analysis would have become normal after applying correctional formulae. In this case with albuminocytological dissociation (CSF collected via LSS), the RBC count was 1470 cells/µl.

The correlations between the CSF parameters and blood contamination were further examined (Table 2). Significant correlations between the blood contamination and the CSF TNCC and CSF protein were found. These correlations were moderate for CSF TNCC and weak for CSF protein concentration. When using the formulae for correction of blood contamination, the correlation between blood contamination and CSF TNCC weakened. To evaluate whether CBC parameters could influence CSF analysis through blood contamination, correlations between CBC WBC and RBC counts and the CSF TNCC and RBC count were assessed using Spearman’s rank correlation coefficient (Table 3). Only a weak correlation between CBC WBC count and CSF TNCC was found.
Long-term follow-up information was collected via telephone conversation with the referring veterinarians of the dogs with abnormal CSF analysis. Follow up was available in 26/30 dogs, with the remaining 4 cases lost to follow up. The median follow-up time for these cases was 32 months (range 12-60 months). The majority of dogs (20/26) showed improvement of the ES frequency with anti-epileptic medication and did not develop any neurological abnormalities. Three other dogs died from unrelated causes namely severe osteoarthritis, leukaemia and sudden death; the latter had been free of ES for 8 months (on maintenance treatment with phenobarbitone) until death but no information was available for the exact time interval for the other two. Finally, the three remaining dogs had poorly controlled ES and were euthanased by the referring veterinarians. One was euthanased 12 months later during status epilepticus (CSF analysis had revealed TNCC of 6 cell/µl and protein concentration of 0.36g/l). The second was euthanased whilst in status epilepticus (CSF analysis had revealed albuminocytological dissociation with CSF protein of 0.31g/l) due to the owner’s request approximately 3 months after discharge. The survival time for the last case was not possible to determine through the referring veterinarian’s records but euthanasia was performed following an episode of status epilepticus; CSF analysis had revealed albuminocytological dissociation with CSF protein of 0.38g/l.

Within our study population, only one dog had a suspected diagnosis different from IE. This patient was diagnosed with suspected metaldehyde intoxication based on the owners’ report of likely exposure and exclusion of other possible causes through the diagnostic investigations (unremarkable haematology and biochemistry profiles, bile acid stimulation, MRI of the brain and CSF analysis) as well as based on the lack of recurrent ES over a follow-up period of 27 months (without receiving antiepileptic medication). In view of these results, the prevalence for a diagnosis other than IE in this study population of dogs was 0.5% (1/200, 95% CI 0.09 – 2.78%); it should be highlighted that CSF analysis in this patient was normal.
Discussion

Dogs that develop ES are often neurologically normal when examined in the interictal period. A Tier II confidence level for the diagnosis of IE is usually desirable and commonly pursued in a referral setting. After exclusion of a systemic disease that might trigger reactive seizures, an MRI study is performed. The likelihood of MR imaging identifying structural abnormalities has been shown to be low in dogs less than 6-years-old (2.2%) but relatively high in those older than that age (26.7%).\(^6\) Obtaining a CSF sample for analysis is commonly performed regardless of the results of previous tests in order to conclusively exclude a possible infectious or inflammatory condition. It should nonetheless be taken into account that this procedure can be associated with significant complications such as iatrogenic brainstem or spinal cord trauma,\(^12\)\(^13\)\(^14\) prolonging anaesthesia time and increasing cost. Our results suggest that performing CSF analysis did not increase the diagnostic sensitivity on a population of epileptic dogs with no interictal abnormalities and unremarkable MRI of the brain.

In 15% of our cases (30 cases), mild abnormalities were found on CSF analysis. Despite this, a diagnosis of IE was made based on otherwise unremarkable diagnostic investigations and lack of interictal neurological abnormalities. Most of these cases (20/26) showed an improvement in the ES frequency and no neurological deficits during a median follow-up time of 32 months. Four cases were lost to follow up, three died due to unrelated causes and three were euthanased due to poorly controlled ES. It was not thought likely but it is possible that some of the patients with CSF abnormalities had a cause for the ES other than IE (either reactive or structural) but without histopathology this is impossible to conclusively establish.

Epileptic seizures have been suggested to cause alterations in the CSF.\(^8\) A previous study\(^8\) reported an association between the CSF TNCC and seizures, with the TNCC tending to decrease as the length of time between the last ES and CSF collection increased. A transient
disturbance of the blood-brain barrier has been demonstrated in experimental animals following ES\textsuperscript{15} and has been suggested as a possible explanation for changes in the composition of CSF following ES in humans\textsuperscript{16}. In our study, we found no association between abnormal CSF protein concentration or TNCC and the time interval between ES episode and CSF collection. These results would suggest that ES are unlikely to significantly alter the composition of CSF. Studies performed in humans revealed that ES-induced CSF abnormalities are uncommon,\textsuperscript{17,18} but mild increases in protein concentration and TNCC can be seen in up to 34% and 10% respectively of human patients after seizures.\textsuperscript{19,20}

Status epilepticus has been found to be more frequently associated with structural epilepsy and reportedly occurred in 28% of IE cases compared to 64% of cases with structural epilepsy.\textsuperscript{21} Single ES versus cluster seizures have been reported as less likely to have an asymmetrical structural lesion of the brain.\textsuperscript{7} In the present study cluster seizures were documented in 56 dogs (28%) and status epilepticus in only 2 dogs (1%). No association between ES semiology (focal or generalised) or occurrence of clusters seizures and the composition of the CSF was found.

CSF does not normally contain erythrocytes. The presence of erythrocytes is most commonly iatrogenic or in some cases associated with a pathological subarachnoid haemorrhage.\textsuperscript{9} Peripheral blood contamination is a common confounding problem in CSF collection independent of technique. The impact of CSF blood contamination is not clear and the current literature on the subject is conflicting. The effect of blood contamination on CSF protein concentration is controversial with some studies showing that samples with RBC counts of 5,000–10,000/µl did not have significantly increased CSF protein\textsuperscript{22,23} whilst others showed a mild but statistically significant increase in CSF samples with RBC counts >500/µl.\textsuperscript{23} Similarly, a significant effect of blood contamination on CSF TNCC has been demonstrated in some studies when RBC counts ranged from 250/µl to 1,500/µl\textsuperscript{24} but not in others.\textsuperscript{25} In our study the number of RBCs on CSF was significantly associated with having an abnormal CSF
analysis. We found that blood contamination was moderately correlated with the CSF TNCC and weakly with the CSF protein concentration. This may have been related to the overall low blood contamination in our study as no association between CSF RBC and protein concentration was found when contamination was <500/µl.23

Several formulae to help estimate the effects of blood contamination on CSF cellularity and protein concentration have been suggested10 11 with the authors commenting that these likely overestimate the effects of peripheral blood contamination and that they may be useful when attempting to confirm disease but should not be used to rule out pathology. When the suggested formulae were used in the present study, the correlation between TNCC and CSF RBC contamination weakened, suggesting that blood contamination does interfere with the CSF analysis results.

This study suggests that performing CSF analysis in dogs with recurrent ES that have a normal interictal neurological examination and unremarkable MRI does not increase the likelihood of detecting an underlying cause.

Acknowledgements

The authors would like to thank all staff and students of the Small Animal Teaching Hospital of the University of Liverpool for keeping the clinical records to the highest possible standards.

References


Table 1. Associations between abnormal CSF analysis and different independent variables

<table>
<thead>
<tr>
<th></th>
<th>Statistic test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of ES:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Generalised or Focal</td>
<td>Fisher’s exact test</td>
<td>p=0.68</td>
</tr>
<tr>
<td>Number of ES:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- ≤5 or &gt;5</td>
<td>Pearson’s Chi-square test</td>
<td>p=0.55</td>
</tr>
<tr>
<td>Cluster ES</td>
<td>Fisher’s exact test</td>
<td>p=0.38</td>
</tr>
<tr>
<td>Status epilepticus</td>
<td>Fisher’s exact test</td>
<td>p=0.28</td>
</tr>
<tr>
<td>Time interval between last ES and CSF analysis</td>
<td>Pearson’s Chi-square test</td>
<td>p=0.92</td>
</tr>
<tr>
<td>CSF collection site:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMC or LSS</td>
<td>Fisher’s exact test</td>
<td>p=0.11</td>
</tr>
<tr>
<td>Number of RBCs on CSF</td>
<td>Mann-Whitney test</td>
<td>p&lt;0.000</td>
</tr>
</tbody>
</table>

CSF - Cerebrospinal Fluid; CMC - Cerebellomedullary Cistern; ES – epileptic seizure; LSS - Lumbar subarachnoid space; RBC - Red Blood Cell.
Table 2. Correlations between CSF parameters and CSF blood contamination

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF TNCC and CSF RBC count</td>
<td>Spearman Rho: 0.427</td>
<td>p&lt;0.000</td>
</tr>
<tr>
<td>Corrected CSF TNCC* and CSF RBC count</td>
<td>Spearman Rho: 0.237</td>
<td>p=0.257</td>
</tr>
<tr>
<td>CSF protein concentration and CSF RBC count</td>
<td>Spearman Rho: 0.253</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Corrected CSF protein concentration§ and CSF RBC count</td>
<td>Spearman Rho: 0.248</td>
<td>p=0.0001</td>
</tr>
</tbody>
</table>

CSF - Cerebrospinal Fluid; TNCC - Total Nucleated Cell Count; RBC - Red Blood Cell.

*CSF TNCC increased by 1 nucleated cell/μl for every 500 RBC/μl

§CSF protein concentration increased by 0.01g/L for every 1,000 RBC/μl of CSF
Table 3. Correlations between CSF blood contamination and CBC parameters

| CBC WBC count and CSF RBC count | Spearman Rho: 0.110 | p=0.160 |
| CBC WBC count and CSF TNCC | Spearman Rho: 0.277 | p=0.025 |
| CBC WBC count and CSF protein concentration | Spearman Rho: 0.135 | p=0.084 |
| CBC RBC count and CSF RBC count | Spearman Rho: -0.029 | p=0.710 |
| CBC RBC count and CSF TNCC | Spearman Rho: -0.120 | p=0.332 |
| CBC RBC count and CSF protein concentration | Spearman Rho: 0.011 | p=0.894 |