1 **Research paper**
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3 **Diagnostic value of cerebrospinal fluid analysis in a population of dogs with suspected**
4 **idiopathic Epilepsy**
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26   **Abstract**

27   Cerebrospinal fluid (CSF) analysis is commonly used in the diagnostic investigation of

28   seizure disorders in order to exclude possible inflammatory underlying aetiology.

29   The medical records were searched for dogs presenting with epileptic seizures (ES) that

30   had normal interictal neurological examination, normal complete blood count and biochemistry

31   analysis, unremarkable magnetic resonance imaging of the brain and had CSF analysis

32   performed as part of the diagnostic investigation.

33   A total of 200 dogs met the inclusion criteria. The CSF was abnormal in 30 dogs with

34   a median total nucleated cell count of 2 cells/µl (IQR 1.5-6) and median protein concentration

35   of 0.37g/l (IQR 0.31-0.41). Pleocytosis was recorded in 14/30 dogs and the CSF protein was

36   increased in 22/30. There was no correlation between abnormal CSF and the type or number of

37   seizures or the time interval between the last seizure and CSF collection. A significant

38   correlation was found between the number of red blood cells on CSF and having an abnormal

39   CSF. The prevalence of having a diagnosis other than suspected idiopathic epilepsy (IE) was

40   0.5% (1/200).

41   These results suggest that performing CSF analysis in dogs with recurrent ES that have

42   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 6y old [6].

67   The value of performing CSF collection and analysis in dogs with suspected IE, after

68 reactive seizures and structural abnormalities on MRI have been excluded, has not yet been

69 determined. The aim of this study was therefore to determine the value of performing CSF

70 analysis in a population of dogs with normal neurological examination in the interictal period,

71 unremarkable haematology and biochemistry and MRI of the brain.

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Materials and methods

73

74   The medical records from the Neurology and Neurosurgery service of the Small Animal

75 Teaching Hospital (SATH) of the University of Liverpool between 2010 and 2015 were

76 reviewed retrospectively to identify dogs presented for investigation of ES. The study protocol

77 was approved by the University of Liverpool's Veterinary Research Ethics Committee

78 (VREC263).

79   Inclusion criteria were that the patients had (1) presented for investigation of ES, (2)

80 unremarkable interictal neurological examination performed by either a board-certified, board

81 eligible neurologists or residents, (3) normal complete blood count (CBC), normal biochemistry

82 profile (or clinically non-significant biochemistry alterations), (4) normal MRI of the brain and

83 (5) cerebrospinal fluid (CSF) analysis performed. Data related to signalment, age at ES onset,

84 ES semiology (focal or generalised), presentation of cluster seizures (defined as 2 or more ES

85 within a 24h period) or status epilepticus (ES which shows no signs of arresting after a duration

86 encompassing the great majority of ES of that type, defined clinically as greater than 5 minutes),

87 and number of ES episodes prior to investigation were documented. The time interval between

88 the last ES and collection of CSF was also recorded. For statistical analysis, the dogs were

89 divided into three groups according to a previous publication[8]: length of time between their last

90 ES and the CSF collection up to 48 hours (group 1); between 3 and 7 days (group 2) and over

91 7 days (group 3).

92   Diagnostic investigation in all cases included a CBC and serum biochemistry profile

93   (sodium, potassium, chloride, calcium, phosphate, alanine aminotransferase, alkaline

94   phosphatise, total bilirubin, urea, creatinine, total protein, albumin, glucose, cholesterol and

95   triglycerides). A bile acid stimulation test and/or fasting ammonia concentration and abdominal

96   ultrasonography were performed when hepatic encephalopathy was clinically suspected.

97   *Neospora caninum* and *Toxoplasma gondii* antibody titres were tested in some cases. Dogs were

98   excluded from the study if corticosteroids had been administered in the 7 days prior to

99   presentation.

100   Magnetic resonance imaging was performed in all cases under general anaesthesia using

101   a 1T (Siemens Magnetom, Erlangen, Germany) scanner. As a minimum, the following

102   sequences were used: T2-weighted images (T2W) (in transverse, sagittal and dorsal planes),

103   fluid attenuation inversion recovery (FLAIR) and pre and post-contrast (intravenous injection

104   of 0.1mmol/kg of gadopentetate dimeglubine) T1-weighted images (T1W) in the transverse

105   plane. A board-certified neurologist (RG) and a board-certified radiologist (TWM) examined

106   all images and dogs with imaging abnormalities were excluded from the study.

107   The CSF collection was performed under the same general anaesthesia from either the

108   cerebellomedullary cistern (CMC) or lumbar subarachnoid space (LSS) after advanced

109   imaging; samples were collected into a plain plastic and EDTA tubes. The analysis was

110   performed within one hour of collection and comprised a red blood cell (RBC) count and total

111   nucleated cell count (TNCC), protein concentration measurement and a cytological

112   examination with a differential cell count. The CSF samples with blood contamination of

113   >5000RBC/μl were excluded from the study.

114   Results of CSF analysis were considered abnormal when the protein concentration was

115   >0.30g/l (CMC) or >0.45g/l (LSS) or the TNCC was >5cells/μl.[9] In the cases where blood

116   contamination was present, formulae for CSF protein concentration and TNCC correction

117   previously reported were used to investigate the possible impact of the blood contamination on

118   CSF analysis: the TNCC was increased by 1 nucleated cell/µl for every 500 RBC/µl[10] and the

119   protein concentration was increased by 0.01g/L for every 1,000 RBC/µl of CSF.[11] The CBC

120   results were also recorded in order to evaluate the influence of the CBC RBC and white blood

121   cell (WBC) counts on the CSF results. The referring veterinarians of the patients that presented

122   abnormal CSF results were contacted via telephone call for a progress update on the long-term

123   outcome of these cases.

124   The sample size calculations indicated a total of 195-245 dogs would be required to

125   estimate the prevalence with a precision of 5% and 95% confidence. All statistical analyses

126   were performed with Minitab 14 (Minitab Inc. State College, Pennsylvania, USA) and R ("The

127   R Project", https://www.r-project.org). Descriptive statistics were computed for variables

128   where appropriate; continuous data were summarised as median values with interquartile ranges

129   (IQR), and categorical data were expressed as frequencies with 95% confidence intervals (95%

130   CI). For categorical variables with a large number of categories and/or categories comprising

131   only small numbers, groups were pooled into appropriate larger classes. For continuous

132   variables normality of distribution was assessed graphically and using the Kolmogorov-

133   Smirnov test. Associations between having an abnormal CSF analysis and categorical variables

134   were assessed using Pearson's Chi square or Fisher's exact test, and associations with

135   continuous variables were assessed using the Mann-Whitney U-test. The relationship between

136   selected continuous variables was evaluated using Spearman's rank correlation coefficient, in

137   order to assess if increases in CSF blood contamination were associated with increases or

138   decreases in CSF parameters or CBC parameters. For all analyses $P<0.05$ was considered

139   significant.

140

141   **Results**

142 A total of 200 dogs with a history of ES and normal interictal neurological examination

143 were included.

144 The CBC was normal in all cases. Biochemical abnormalities were found in 6 dogs and

145 were mainly related to mild increment in liver enzymes (particularly serum alkaline

146 phosphatase); 3/6 cases were receiving phenobarbitone. Serology antibody titres for

147 *Toxoplasma gondii* and *Neospora caninum* were performed in 23/200 (11.5%) cases and were

148 negative in all cases.

149 The majority of dogs were male (62.5%). The most prevalent breeds were: crossbreed

150 (14.5%), Labrador retriever (8.5%), Border collie (8.5%) and Staffordshire bull terrier (5.5%).

151 Median age at ES onset was 43.5 months (IQR 25-154) with 22 dogs (11%) <1y old, 130 dogs

152 (65%) between 1y-6y and 48 dogs (24%) >6y old. Sixty-seven dogs (33.5%) presented focal

153 ES and 133 dogs (66.5%) generalised ES according to the owners' description. Cluster seizures

154 were reported in 57 dogs (28.5%) and only 2 dogs (1%) had a previous episode of status

155 epilepticus. Of the total population, 90 (45%) dogs suffered from ≤ 5 ES episodes prior to

156 investigations and 110 (55%) of dogs had > 5 ES episodes recorded. The time interval between

157 CSF collection and last ES episode was <2 days in 45 dogs (22.5%); between 3-7 days in 55

158 dogs (27.5%) and >7 days in 100 cases (50%).

159 The CSF samples were collected via CMC in 196 cases (98%) and LSS in 4 cases (2%).

160 The median CSF RBC count was 1.5 (IQR 1.5-40) cells/µl, median TNCC was 1.5 cells/µl

161 (IQR 1.5-2) and median protein concentration was 0.2 g/l (IQR 0.17- 0.26).

162 The CSF analysis was abnormal in 30 dogs (15%, 95% CI 0.11 – 0.21). For the abnormal

163 cases, the median CSF RBC count was 40 cells/µl (IQR 2-360), median TNCC was 2 cells/µl

164 (IQR 1.5-6) and median protein concentration was 0.37g/l (IQR 0.31-0.41). Of these, 1 dog had

165 a history of status epilepticus and 6 dogs had cluster seizures reported. In 14/30 cases

166 pleocytosis was identified with a median TNCC of 6 cells/µl (IQR 6-8). The samples were

167 collected from CMC in 13 dogs and in 1 dog from the LSS. The differential cell count of patients

168 with pleocytosis revealed mononuclear predominance in all cases.

169 The CSF protein was increased in 22/30 dogs of which 21 had been sampled from the

170 CMC and 1 from the LSS. The median value for abnormal CSF protein concentration collected

171 from the CMC was 0.4g/l (IQR 0.36-0.42) and 0.46g/l on the case collected from the LSS. Both

172 pleocytosis and raised CSF protein concentration were present in 6/30 cases and

173 albuminocytological dissociation was present in 16/30 dogs.

174 The majority of dogs (16/30) with abnormal CSF had a time interval between the last

175 ES and CSF analysis >7 days and 8/30 had CSF collected <2 days since the last ES. Twelve

176 dogs with abnormal CSF analysis had suffered more than 5 ES prior to examination.

177 Associations between having an abnormal CSF and multiple independent variables were

178 assessed (Table 1). The only significant correlation found was between the number of RBCs on

179 CSF and having an abnormal CSF result (Mann-Whitney Test p<0.000). In only one of these

180 cases the CSF analysis would have become normal after applying correctional formulae. In this

181 case with albuminocytological dissociation (CSF collected via LSS), the RBC count was 1470

182 cells/µl.

183 The correlations between the CSF parameters and blood contamination were further

184 examined (Table 2). Significant correlations between the blood contamination and the CSF

185 TNCC and CSF protein were found. These correlations were moderate for CSF TNCC and

186 weak for CSF protein concentration. When using the formulae for correction of blood

187 contamination, the correlation between blood contamination and CSF TNCC weakened. To

188 evaluate whether CBC parameters could influence CSF analysis through blood contamination,

189 correlations between CBC WBC and RBC counts and the CSF TNCC and RBC count were

190 assessed using Spearman's rank correlation coefficient (Table 3). Only a weak correlation

191 between CBC WBC count and CSF TNCC was found.

192   Long-term follow-up information was collected via telephone conversation with the

193   referring veterinarians of the dogs with abnormal CSF analysis. Follow up was available in

194   26/30 dogs, with the remaining 4 cases lost to follow up. The median follow-up time for these

195   cases was 32 months (range 12-60 months). The majority of dogs (20/26) showed improvement

196   of the ES frequency with anti-epileptic medication and did not develop any neurological

197   abnormalities. Three other dogs died from unrelated causes namely severe osteoarthritis,

198   leukaemia and sudden death; the latter had been free of ES for 8 months (on maintenance

199   treatment with phenobarbitone) until death but no information was available for the exact time

200   interval for the other two. Finally, the three remaining dogs had poorly controlled ES and were

201   euthanased by the referring veterinarians. One was euthanased 12 months later during status

202   epilepticus (CSF analysis had revealed TNCC of 6 cell/μl and protein concentration of 0.36g/l).

203   The second was euthanased whilst in status epilepticus (CSF analysis had revealed

204   albuminocytological dissociation with CSF protein of 0.31g/l) due to the owner's request

205   approximately 3 months after discharge. The survival time for the last case was not possible to

206   determine through the referring veterinarian's records but euthanasia was performed following

207   an episode of status epilepticus; CSF analysis had revealed albuminocytological dissociation

208   with CSF protein of 0.38g/l.

209   Within our study population, only one dog had a suspected diagnosis different from IE.

210   This patient was diagnosed with suspected metaldehyde intoxication based on the owners'

211   report of likely exposure and exclusion of other possible causes through the diagnostic

212   investigations (unremarkable haematology and biochemistry profiles, bile acid stimulation,

213   MRI of the brain and CSF analysis) as well as based on the lack of recurrent ES over a follow-

214   up period of 27 months (without receiving antiepileptic medication). In view of these results,

215   the prevalence for a diagnosis other than IE in this study population of dogs was 0.5% (1/200,

216   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

242 disturbance of the blood-brain barrier has been demonstrated in experimental animals following

243 ES[15] and has been suggested as a possible explanation for changes in the composition of CSF

244 following ES in humans[16]. In our study, we found no association between abnormal CSF protein

245 concentration or TNCC and the time interval between ES episode and CSF collection. These

246 results would suggest that ES are unlikely to significantly alter the composition of CSF. Studies

247 performed in humans revealed that ES-induced CSF abnormalities are uncommon,[17][18] but mild

248 increases in protein concentration and TNCC can be seen in up to 34% and 10% respectively

249 of human patients after seizures.[19][20]

250 Status epilepticus has been found to be more frequently associated with structural epilepsy and

251 reportedly occurred in 28% of IE cases compared to 64% of cases with structural epilepsy.[21]

252 Single ES versus cluster seizures have been reported as less likely to have an asymmetrical

253 structural lesion of the brain.[7] In the present study cluster seizures were documented in 56 dogs

254 (28%) and status epilepticus in only 2 dogs (1%). No association between ES semiology (focal

255 or generalised) or occurrence of clusters seizures and the composition of the CSF was found.

256 CSF does not normally contain erythrocytes. The presence of erythrocytes is most

257 commonly iatrogenic or in some cases associated with a pathological subarachnoid

258 haemorrhage.[9] Peripheral blood contamination is a common confounding problem in CSF

259 collection independent of technique. The impact of CSF blood contamination is not clear and

260 the current literature on the subject is conflicting. The effect of blood contamination on CSF

261 protein concentration is controversial with some studies showing that samples with RBC counts

262 of 5,000–10,000/µl did not have significantly increased CSF protein[22][23] whilst others showed

263 a mild but statistically significant increase in CSF samples with RBC counts >500/µl.[23]

264 Similarly, a significant effect of blood contamination on CSF TNCC has been demonstrated in

265 some studies when RBC counts ranged from 250/µl to 1,500/µl [24] but not in others.[25.] In our

266 study the number of RBCs on CSF was significantly associated with having an abnormal CSF

267 analysis. We found that blood contamination was moderately correlated with the CSF TNCC

268 and weakly with the CSF protein concentration. This may have been related to the overall low

269 blood contamination in our study as no association between CSF RBC and protein

270 concentration was found when contamination was <500/µl.[23]

271   Several formulae to help estimate the effects of blood contamination on CSF cellularity

272 and protein concentration have been suggested[10] [11] with the authors commenting that these

273 likely overestimate the effects of peripheral blood contamination and that they may be useful

274 when attempting to confirm disease but should not be used to rule out pathology. When the

275 suggested formulae were used in the present study, the correlation between TNCC and CSF

276 RBC contamination weakened, suggesting that blood contamination does interfere with the

277 CSF analysis results.

278   This study suggests that performing CSF analysis in dogs with recurrent ES that have a

279 normal interictal neurological examination and unremarkable MRI does not increase the

280 likelihood of detecting an underlying cause.

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

	Statistic test	p-value
Type of ES: - Generalised or Focal	Fisher's exact test	p=0.68
Number of ES: - ≤ 5 or >5	Pearson's Chi-square test	p=0.55
Cluster ES	Fisher's exact test	p=0.38
Status epilepticus	Fisher's exact test	p=0.28
Time interval between last ES and CSF analysis	Pearson's Chi-square test	p=0.92
CSF collection site: CMC or LSS	Fisher's exact test	p=0.11
Number of RBCs on CSF	Mann-Whitney test	p<0.000

378
379   CSF - Cerebrospinal Fluid; CMC - Cerebellomedullary Cistern; ES – epileptic seizure; LSS -
380   Lumbar subarachnoid space; RBC - Red Blood Cell.

381   Table 2. Correlations between CSF parameters and CSF blood contamination

	Correlation coefficient	p-value
CSF TNCC and CSF RBC count	Spearman Rho: 0.427	p<0.000
Corrected CSF TNCC∗ and CSF RBC count	Spearman Rho: 0.237	p=0.257
CSF protein concentration and CSF RBC count	Spearman Rho: 0.253	p=0.0001
Corrected CSF protein concentration§ and CSF RBC count	Spearman Rho: 0.248	p=0.0001

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

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

384   §CSF protein concentration increased by 0.01g/L for every 1,000 RBC/μl of CSF

385 Table 3. Correlations between CSF blood contamination and CBC parameters

	Correlation coefficient	p-value
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

386
387 CSF - Cerebrospinal Fluid; TNCC - Total Nucleated Cell Count; RBC - Red Blood Cell; WBC
388 - White Blood Cell.