

Comparison of serum creatine kinase and aspartate aminotransferase activity in dogs with *Neospora* meningoencephalitis and noninfectious meningoencephalitis

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Abstract

Background: Creatine kinase (CK) and aspartate aminotransferase (AST) activity can be increased with myositis associated with *Toxoplasma* and *Neospora* infection in dogs.

Hypothesis/Objectives: Serum activity of CK and AST can be used as a rapid screen for predicting positive serology in meningoencephalitis caused by *Toxoplasma gondii* or *Neospora caninum* in dogs compared to dogs with noninfectious meningoencephalitis.

Animals: Eighty dogs with meningoencephalitis based on magnetic resonance imaging and cerebrospinal fluid analysis.

Methods: Retrospective case-control study. Serological cutoffs ($\geq 1:800$ immunofluorescence for *Neospora* and $\geq 1:400$ IgG or $\geq 1:64$ IgM or both for *Toxoplasma*) categorized dogs as infected ($n = 21$, all neosporosis) or noninfected ($n = 59$). Activities of CK and AST between infected and noninfected groups were compared using a Mann-Whitney *U* test and receiver operating characteristic curve analysis.

Results: No dogs were diagnosed with toxoplasmosis. Serum CK and AST activities were significantly increased ($P < .001$) in dogs with positive serology for *Neospora* (CK: median, 1334 U/L; range, 281–3633 U/L and AST: median, 124 U/L; range, 59–333 U/L) compared to noninfectious cases (CK: median, 215 U/L; range, 69–683 U/L and AST: median, 36 U/L; range, 19–139 U/L). A CK cutoff of 485 U/L had 95.24% sensitivity and 96.61% specificity with a negative predictive value of >99%. An AST cutoff of 57 U/L had 94.44% sensitivity and 85.71% specificity with an estimated negative predictive value of 99%.

Conclusions and Clinical Importance: High serum CK and AST activity can increase suspicion for neosporosis while awaiting serological tests for dogs with meningoencephalitis.

KEYWORDS

dog, meningoencephalitis, meningoencephalitis of unknown origin, *Neospora*

Abbreviations: AST, aspartate aminotransferase; CI, confidence interval; CK, creatine kinase; CNS, central nervous system; CSF, cerebrospinal fluid; IFA, immunofluorescence; MRI, magnetic resonance imaging; MUO, meningoencephalitis of unknown origin; NPV, negative predictive value; PCR, polymerase chain reaction; ROC, receiver operating characteristic curve; TNCC, total nucleated cell count.

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1 | INTRODUCTION

Meningoencephalitis of unknown origin (MUO) is a general term for a group of clinically diagnosed inflammatory brain diseases for which histopathological confirmation of the underlying cause is lacking. Diagnosis is presumptive, based on neurological examination findings, magnetic resonance imaging (MRI) changes, and cerebrospinal fluid (CSF) pleocytosis (total nucleated cell count [TNCC] >5 nucleated cells/ μ L with >50% mononuclear cells)¹ and once negative serological testing for infectious diseases, particularly *Toxoplasma* and *Neospora* in the United Kingdom, is obtained.¹ The pathogenesis of MUO is unclear. It is hypothesized that an autoimmune process is involved^{2,3} and treatment therefore has been aimed at immunosuppression.^{4,5} Prognosis is variable with median survival times ranging from 0 to 602 days^{6,7} and one-third of dogs deteriorating within the first 72 hours after treatment.⁶

Toxoplasma and *Neospora* are 2 infectious protozoa that can cause central nervous system (CNS) disease but also other systemic disease, particularly myositis, because the organisms encyst in the skeletal muscle of intermediate hosts such as the dog.^{8,9} Seroprevalence in dogs with meningoencephalitis in the United Kingdom is reported to be low (3.98% and 0.25% for exposure and active clinical infection respectively for *Toxoplasma* and 6.96% and 2.25% for *Neospora*).¹⁰ The neurological and diagnostic imaging findings in protozoal encephalitis are similar to those of MUO.^{8,9} Definitive diagnosis is based on the identification of the protozoa in CSF or tissues,⁸ doing so is often difficult. Clinical suspicion of active infection in dogs with neurological signs can be supported by positive serology antibody titers of >1:400 IgG or >1:64 IgM or both for *Toxoplasma*,^{11,12} \geq 1:800 immunofluorescence (IFA) for *Neospora*,¹³⁻¹⁵ or polymerase chain reaction (PCR) on CSF.¹⁶⁻¹⁸ Serological and PCR testing is associated with a delay in treatment and risk of immunosuppressing dogs with active clinical infection.

Creatine kinase (CK) and aspartate aminotransferase (AST) are enzymes primarily found in the cytoplasm of skeletal muscle cells and other tissues such as the liver.^{19,20} An increase in the activity of these enzymes in serum may be an indicator of muscle damage.^{21,22}

Our aim was to evaluate the use of CK and AST as indicators to increase suspicion for active clinical infection while awaiting serological test results.

We hypothesized that CK and AST activity would be increased in dogs with positive serology, PCR, or both for *Toxoplasma* or *Neospora* in comparison to dogs with MUO, even in the absence of overt clinical signs of myositis.

2 | METHODS

The clinical database of a referral teaching hospital in the United Kingdom (Langford Veterinary Services, Bristol) was retrospectively reviewed for dogs with presumed meningoencephalitis (infectious and noninfectious) between 2013 and 2020.

Dogs were included in the study if they met the following criteria based on the guidelines from a meta-analysis on noninfectious

meningoencephalitis¹: (a) neurological signs compatible with focal or multifocal CNS disease and (b) either MRI changes or CSF pleocytosis (TNCC >5 nucleated cells/ μ L with >50% mononuclear cells) compatible with presumed meningoencephalitis, and (c) *Toxoplasma* and *Neospora* serology performed on a sample taken at the time of diagnosis with or without PCR on CSF. In addition, we only included dogs with serum CK and AST activities measured within 12 hours of MRI and CSF sampling.

Dogs were categorized as infected or noninfected based on serology \geq 1:800 IFA or positive PCR for *Neospora* or both and >1:400 IgG or >1:64 IgM or both for *Toxoplasma*. Serological testing was performed using an IFA technique with starting screening dilutions of 1:100 for *Neospora* IgG and 1:50 for *Toxoplasma* IgG and 1:25 for IgM.

If requested, quantitative PCR was performed on CSF samples for the detection of *Neospora caninum* and *Toxoplasma gondii* using *N caninum* Nc5 marker genomic sequence assays and Genesig *T gondii* repeat region, respectively. Assays were performed by Langford Vets Diagnostic Lab on a Qtower3 (Analytik Jena, Germany) following a laboratory-validated thermocycling protocol.

Dogs were excluded if they had a previous diagnosis of toxoplasmosis or neosporosis before presentation to the referral hospital, a previous history of myositis, trauma within 48 hours of presentation, or were unable to move or walk without assistance.

2.1 | Statistical analysis

Data were analyzed using the commercially available statistical program GraphPad© and examined for normality by visual assessment of a quantile plot and the Shapiro-Wilk test. Data were not normally distributed and descriptive statistics were expressed as median (range). A Mann-Whitney *U* test was used to compare CK and AST activities between infected and noninfected groups. Statistical significance was set at $P < .05$ and all analyses were 2-tailed.

A receiver operating characteristic curve (ROC) analysis was performed to calculate the sensitivities and specificities that corresponded to a range of CK and AST activities using positive serology as a gold standard.

The cutoff serum activities for CK and AST with the highest likelihood ratios then were used to calculate positive predictive value and negative predictive value (NPV) using prevalence data for active protozoal infection in dogs in the United Kingdom with meningoencephalitis.¹⁰

3 | RESULTS

A total of 161 dogs with meningoencephalitis were identified, of which 80 had CK and AST activities measured at the time of diagnosis. Twenty-one dogs (26%) had positive serology for *Neospora*; 19/21 dogs had positive serology of >1:1600 and 2 dogs had positive titers of >1:800. Polymerase chain reaction on CSF was performed in 17 of the 21 dogs with positive serology and 9 were positive, all for

Neospora. Seven of the 9 dogs with positive PCR had titers >1:1600 and 1 dog had a titer >1:3200. A protozoal cyst was identified in a muscle biopsy sample in 1 dog.

No dogs were identified with positive serology or PCR or both for Toxoplasma.

Fifty-nine dogs were diagnosed with MUO based on negative serology and PCR for Neospora. Of the 59 MUO cases, 8/59 dogs had seizures within 24 hours of presentation. One dog also presented with muscle tremors.

Dogs with a positive serology (with or without PCR) for Neospora had higher CK activity (median, 1334 U/L; range, 281-3633 U/L) compared to noninfectious cases (median, 215 U/L; range, 69-683 U/L; $P < .001$; Figure 1A).

The ROC analysis for CK is shown in Figure 1B with an area under the curve value of 0.9798 (95% confidence interval [CI], 0.9493-1.000).

Sensitivity and specificity values for CK generated from the ROC analysis are shown in Table 1. A CK cutoff of 485 U/L gave the highest likelihood ratio (28.10) to predict serological diagnosis of Neospora with a sensitivity of 95.24% (95% CI, 0.7733-0.9976) and specificity of 96.61% (95% CI, 0.8846-0.9940). Using a prevalence of 2.25% for active infection with Neospora in the United Kingdom,¹⁰ we calculated a negative predictive value of 99%, suggesting that dogs with CK <485 U/L are very unlikely to have a serological diagnosis of Neospora. Twenty of

21 dogs with positive serology for Neospora had CK activity >485 U/L with 1 dog having CK activity of 285 U/L.

Serum CK activities were above the identified cutoff of >485 U/L in 2 dogs (2/59) with MUO.

The activity of AST also was increased in dogs with positive serology for Neospora (median, 124 U/L; range, 59-333 U/L) compared to cases of MUO (median, 36 U/L; range, 19-139; $P < .001$; Figure 2). The ROC analysis for AST is shown in Figure 1B with an area under the curve of 0.9518 (95% CI, 0.9060-0.9977).

The sensitivity and specificity values for AST generated from the ROC analysis are shown in Table 2. An AST cutoff of 57.50 U/L gave the highest likelihood ratio (6.61) to predict serological diagnosis of Neospora with a sensitivity of 94.44% (95% CI, 0.7424-0.9972) and specificity 85.71% (95% CI, 0.7333-0.9290) with a negative predictive value of 99%. Twenty of 21 dogs with positive Neospora serology had AST activity >57.50 U/L with 1 dog having an activity of 50 U/L. This dog was the same dog with CK activity of 285 U/L.

Nine dogs (9/59) with MUO had serum AST activity above the identified cutoff of 57.50 U/L.

Dogs with MUO that had seizures within 24 hours of presentation had median CK activity of 342.5 U/L (range, 190-683 U/L) and median AST activity of 53.14 U/L (range, 34-83 U/L), lower than the cutoff selected from the ROC analysis. One dog with seizures and

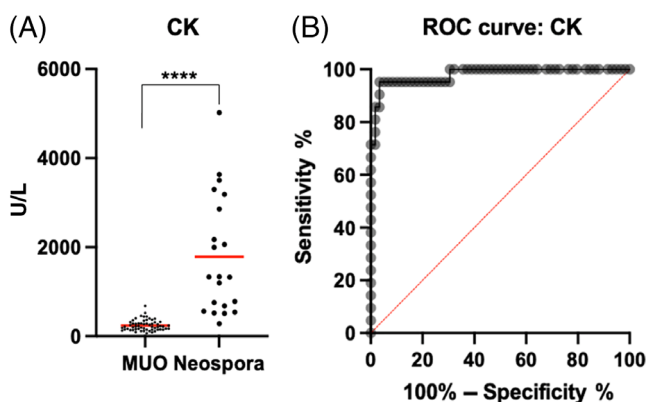


FIGURE 1 (A) CK activity in dogs with positive serology for Neospora compared with negative serology and suspected noninfectious meningoencephalitis (**** $P < .001$). (B) ROC analysis for CK. CK, creatine kinase; MUO, meningoencephalitis of unknown origin; ROC, receiver operating characteristic

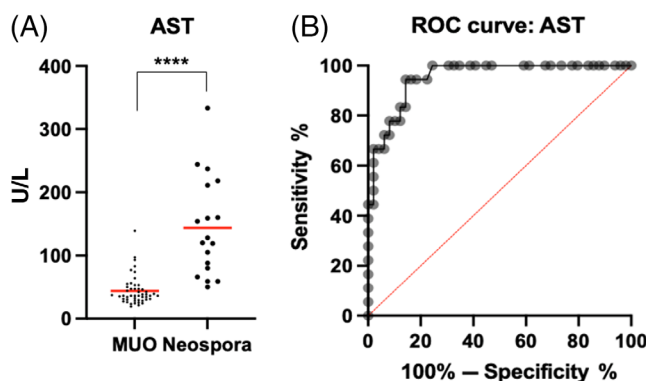


FIGURE 2 (A) AST activity in dogs with positive serology for Neospora compared with negative serology and suspected noninfectious meningoencephalitis (**** $P < .001$). (B) ROC analysis for AST. AST, aspartate aminotransferase; MUO, meningoencephalitis of unknown origin; ROC, receiver operating characteristic

TABLE 1 Sensitivity and specificity for CK activity generated from ROC analysis to calculate negative predictive value (NPV)

Serum CK cutoff activity (reference 75-230 U/L)	Sensitivity %	95% CI	Specificity %	95% CI	Likelihood ratio	NPV (prevalence 2.25%)
>450.0	95.24	77.33%-99.76%	94.92	86.08%-98.61%	18.73	0.999
>485.0	95.24	77.33%-99.76%	96.61	88.46%-99.40%	28.10	0.999
>517.0	90.48	71.09%-98.31%	96.61	88.46%-99.40%	26.69	0.997
>522.5	85.71	65.36%-95.02%	96.61	88.46%-99.40%	25.29	0.996

Abbreviations: CI, confidence interval; CK, creatine kinase; ROC, receiver operating characteristic.

TABLE 2 Sensitivity and specificity for AST activity generated from ROC analysis to calculate negative predictive value (NPV)

Serum AST cutoff activity (reference 20-35 U/L)	Sensitivity %	95% CI	Specificity %	95% CI	Likelihood ratio	NPV (prevalence 2.25%)
>54.00	94.44	74.24%-99.72%	81.63	68.64%-90.02%	5.142	0.998
>55.50	94.44	74.24%-99.72%	83.67	70.96%-91.49%	5.785	0.998
>57.50	94.44	74.24%-99.72%	85.71	73.33%-92.90%	6.611	0.998
>61.50	83.33	60.78%-94.16%	85.71	73.33%-92.90%	5.833	0.995

Abbreviations: AST, aspartate aminotransferase; CI, confidence interval; ROC, receiver operating characteristic.

negative serology had CK activity of 683 U/L and AST 74 U/L, which may have accounted for the moderate increase in this case.

4 | DISCUSSION

We highlighted the clinical dilemma when presented with a dog with multifocal CNS signs while awaiting serological testing for infectious diseases. Using CK and AST activities measured at the time of diagnosis, we assessed the ability to predict an abnormal serological result. We found that serum activities of CK and AST were increased in dogs with infectious meningoencephalitis associated with *Neospora*. High sensitivity and NPV suggest that measuring CK activity at the time of MRI and CSF sampling would be an effective tool to screen for neosporosis in dogs with meningoencephalitis of uncertain origin.

We did not find any dogs with positive serology (>1:400 IgG or >1:64 IgM or both) with or without PCR for *Toxoplasma*, which likely reflects the low seroprevalence for active clinical infection of 0.25% reported in the United Kingdom.¹⁰

Receiver operating characteristic curve analysis was used to evaluate the use of CK and AST in predicting abnormal serology using sensitivity as a function of the false positive rate for different cutoffs. We focused on the NPV for a range of cutoffs to assess the reliability for using CK and AST as markers to distinguish between normal and abnormal serological testing. Using the cutoffs identified for CK and AST, we may have risked instituting immunosuppression for MUO in 1 dog with positive serology for *Neospora* and delayed treatment in 2 dogs with MUO. However, the clinical consequences of doing so are not well established, and some affected dogs may benefit from anti-inflammatory doses of corticosteroids because of the severity of inflammation that can be associated with *Neospora* infection, whereas previous experimental studies have reported worsening of clinical neosporosis with the use of corticosteroids.²³

One dog with positive serology for *Neospora* (>1:1600) had CK and AST activities (285 U/L and 50 U/L, respectively) that were lower than the ROC cutoffs for both enzymes with the highest NPV. We could have risked immunosuppressing this dog if we only used the cutoff values identified, but the clinical presentation was strongly suggestive for *Neospora*. This dog had multifocal neurological signs (cerebellovestibular and thoracolumbar spinal cord), decreased cerebellar volume (decreased volume of parenchyma with prominent sulci and widening of the folia and increased volume of CSF) identified on MRI, and a mixed pleocytosis

of 7 cells/ μ L on CSF analysis. Possible reasons for the mild increase in CK and AST activities include inadequate timing of the sample because of the short half-life of the enzymes (2-4 hours for CK, 22 hours for AST), an early stage in the clinical disease not yet causing clinically relevant muscle damage, or equivocal clinical infection because of lack of histopathological confirmation. The enzymes CK and AST are not entirely specific for skeletal muscle and can be found other tissues such as myocardium and intestine and AST is also present in the liver.¹⁹ The main source of serum CK is skeletal muscle²⁴ whereas AST is less specific for skeletal muscle than CK because of its presence in the liver, and serum activity can increase with liver damage.²⁰ We did not investigate the combination of clinical features and serum CK and AST activity, but future research to improve the accuracy of CK and AST serum activity as a screening test is warranted.

We aimed to exclude dogs with that were recumbent, had a previous history of myositis or recent trauma because such dogs would be expected to have higher activities of CK and AST from muscle damage that would be less likely to be directly related to infection with *Toxoplasma* or *Neospora*. Similarly, we could have excluded dogs with seizures and muscle tremors because these have an impact on CK and AST activity. A study in humans showed that CK activity was increased in 82% of patients with generalized seizures.²⁵

Because seizures are a typical presentation for patients with fore-brain disease and have not been shown to be more or less common in MUO than in protozoal encephalitis, we felt that including dogs with recent seizures was appropriate because doing so accurately reflected the population of interest.

The main limitations of our study include its retrospective nature and that the case selection process will have inadvertently introduced bias because of the lack of histopathological confirmation of active clinical infection. In our cohort, we did not find any dogs that had positive PCR with negative serology. The sensitivity of PCR is reported to be higher than that of serological testing,²⁶ but it relies on the protozoa being present in the CSF at the time of sampling. Therefore, it could be possible to have negative PCR results in dogs that have not yet seroconverted. Furthermore, serological testing does not correlate well with PCR, and serology can underestimate positive cases infected with *Neospora*.¹⁸

In conclusion, serum CK and AST activity can be used to aid the clinical decision-making process when presented with a dog with suspected meningoencephalitis that requires urgent treatment before serological testing. Additional studies are required to quantify these findings in a larger population of dogs and to confirm active clinical

infection with histopathology where possible and to evaluate if other clinical information can improve diagnostic accuracy.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the University of Bristol ethical committee VIN/21/009.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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