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Original Research Article

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Serum Neuro-Injury and Redox Markers in Dogs with Suspected Distemper

Key words

anti-MOG;
biomarkers;
CDV;
central nervous system damage;
GFAP;
NFL;
oxidative stress

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Abstract: This study aimed to investigate central nervous system damage and oxidative stress in dogs brought to clinics and hospitals in Ankara, Türkiye with suspected distemper using the same sample. 19 dogs confirmed suspected positive Canine Distemper Virus (CDV+) with lateral flow immunochromatographic rapid test kits formed the 'CDV+' group, while 23 clinically healthy dogs with negative test results formed the 'control' group. The goal is to identify practical, serum-based markers that can be used for early diagnosis, prognosis determination, and monitoring treatment response in distemper. Glial fibrillary acidic protein (GFAP) levels were significantly higher in the CDV+ group compared to the control group ($p = 0.011$; False Discovery Rate (FDR-p) = 0.025), whereas Total oxidant status (TOS) and oxidative stress index (OSI) were unexpectedly low (FDR-p = 0.011 for both). No statistical differences were found in neurofilament light chain (NFL), myelin oligodendrocyte glycoprotein antibody (Anti-MOG), total antioxidant status (TAS), and malondialdehyde (MDA). In receiver operating characteristic (ROC) analysis, the area under the curve (AUC) for GFAP was 0.73, while TOS and OSI (interpreted inversely as indicating low-value disease) yielded AUC values of 0.78 and 0.79, respectively. At the best thresholds, GFAP ≥ 460 ng/L yielded 68% sensitivity and 74% specificity, while TOS ≤ 333 µmol/L and OSI ≤ 12 au thresholds yielded 91% specificity. The multiple logistic model (AUC = 0.86) increased sensitivity and specificity to 84% and 83%, respectively; however, only GFAP was a significant independent predictor (Odds Ratio (OR) ≈ 4.1; $p = 0.048$). In conclusion, serum GFAP appears to reflect central nervous system (CNS) involvement in dogs infected with distemper, and the combination of GFAP with low TOS and OSI values may enhance diagnostic specificity. These findings indicate that GFAP-based, oxidative marker-inclusive multi-biomarker panels could hold potential for supporting the early diagnosis and prognosis monitoring of canine distemper, although further validation in larger and longitudinal studies is warranted.

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Introduction

Canine distemper virus (CDV) is a single-stranded RNA virus belonging to the *Morbillivirus* genus and causes a multisystemic infection with high morbidity and mortality in dogs and many wild species (1, 2). The disease typically begins with respiratory, gastrointestinal, and dermatological symptoms and often progresses to a neurological form characterized by encephalomyelitis, optic nerve degeneration, and demyelination, potentially leading to permanent neurological sequelae or death (3). The absence of specific clinical signs in the early phase, stage-dependent viral tropism, and the limited diagnostic value of routine hematological and biochemical tests have increased the need for sensitive and rapidly applicable serum biomarkers for distemper (4).

In recent years, it has been demonstrated that proteins of central nervous system (CNS) origin can be measured in peripheral blood, providing insights into neurodegenerative processes (5). Glial fibrillary acidic protein (GFAP), indicative of astrocytic damage, and neurofilament light chain (NFL), a marker of axonal integrity, have shown diagnostic potential in human medicine for conditions

such as traumatic brain injury, multiple sclerosis, and viral encephalitis, and in veterinary medicine for meningoencephalitis and epileptic disorders (6, 7). However, peripheral GFAP and NFL data in distemper are limited and results are heterogeneous.

The autoimmune response to neuronal antigens is also notable in the immunopathogenesis of CDV infection. In particular, antibodies against myelin oligodendrocyte glycoprotein (MOG) may contribute to the development of demyelinating lesions, although evidence regarding the diagnostic value of Myelin Oligodendrocyte Glycoprotein Antibody (Anti-MOG) in field cases is limited (8).

During infection, mechanisms such as cytokine storm, mitochondrial dysfunction, and radical production lead to an increase in cellular oxidative load. Total oxidant status (TOS), total antioxidant status (TAS), and their ratio, calculated as the oxidative stress index (OSI), quantitatively reflect the organism's net oxidant–antioxidant balance (9). Although significant alterations in these parameters have been reported in various viral diseases, data on TOS, TAS, OSI, and malondialdehyde (MDA), a product of lipid peroxidation, in distemper are inconsistent; the number of studied cases is often low, and neurological and systemic forms are generally not distinguished (10).

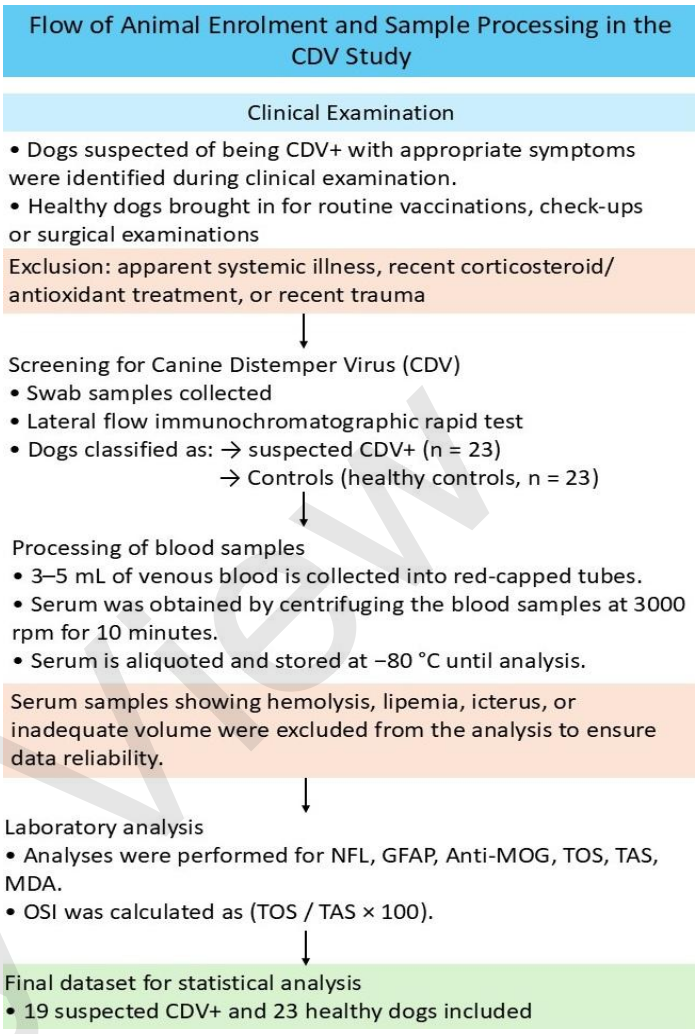
Based on these knowledge gaps, this study aims to confirm the presence of CDV in dogs with distemper using lateral flow immunochromatographic rapid antigen tests, to compare serum levels of GFAP, NFL, Anti-MOG, TOS, TAS, OSI, and MDA with those of healthy dogs, to describe the neurological and oxidative biomarker profile of distemper, and to determine the diagnostic performance of single and multiple biomarker models. Ultimately, the goal is to identify practical, serum-based indicators that can support early diagnosis, prognosis assessment, and treatment monitoring in canine distemper.

Material and methods

Study Design and Case Selection

This exploratory pilot study was conducted between February and May 2025 and included dogs brought for examination to six independent veterinary clinics/animal hospitals in Ankara. 19 dogs suspected of distemper were included in the "suspected CDV+" group based on clinical signs (fever, nasal-ocular discharge, coughing, myoclonus, etc.) and positive lateral flow immunochromatographic rapid CDV antigen test results. In the same period, 23 clinically healthy dogs brought in for routine vaccination or surgical check-up and testing negative on CDV rapid tests comprised the "Control" group. The animals to be included in the study were determined using G power analysis. Dogs with apparent systemic illness, recent corticosteroid/antioxidant treatment, or recent trauma were excluded from the study. Serum samples showing hemolysis, lipemia, icterus, or inadequate volume were excluded from the analysis to ensure data reliability (Figure 1).

Figure 1: Workflow of Clinical, Laboratory, and Analytical Procedures in the CDV Study



NFL= Neurofilament Light Chain; GFAP= Glial Fibrillary Acidic Protein; Anti-MOG= Myelin Oligodendrocyte Glycoprotein Antibody; TOS= Total Oxidant Status. TAS= Total Antioxidant Status; OSI= Oxidative Stress Index; MDA= Malondialdehyde; CDV= Canine Distemper Virus; n= sample size

Ethical Approval

The study protocol was approved by the Local Ethics Committee for Animal Experiments of Kırıkkale University (Decision No. 2025-01-11), and informed consent was obtained from all animal owners.

Sample Collection and Rapid Testing

After collecting anamnesis, a full physical examination was performed on all dogs. For CDV antigen screening, samples were obtained from rectal swabs and nasal discharge and tested using a commercial lateral flow immunochromatographic rapid antigen test kit (Canivet Canine Distemper Ag test Cat. No VD027) in accordance with the manufacturer's protocol. From each dog confirmed suspected CDV+ or classified as control, 2 mL of whole blood was collected from the cephalic vein into a single tube. Following clotting, the samples were centrifuged at 4000 g for 10 minutes at +4 °C to separate serum, which was stored

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at –20 °C until analysis. One limitation of this study is the absence of PCR confirmation for CDV diagnosis, as PCR was not routinely available in the participating clinics during the enrolment period. This may slightly reduce the specificity of case classification.

Measurement of Neurological Biomarkers

GFAP, NFL, and Anti-MOG concentrations were determined using species-specific BT-Lab ELISA kits (Cat. No: E0499Ca, E0492Ca, ED0042Ca), based on the double-antibody sandwich principle described in the manufacturer's instructions. Absorbance was read at 450 nm using a Bio-Tek EL x 800 microplate reader, with background correction by subtracting blank well values. The analytical sensitivity values reported by the manufacturers were 1.03 µg/L for NFL and 0.85 ng/L for GFAP. The intra-assay and inter-assay coefficients of variation (CV) were < 8 % and < 10 % for NFL and GFAP, and < 10 % and < 12 % for Anti-MOG, respectively. Sensitivity and specificity data were not provided in the manufacturer's documentation.

Oxidative Stress Panel

Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) were measured using colorimetric kits from Rel Assay Diagnostics (Turkey) (Cat. No: RL0017, RL0024), based on changes in the color of ABTS radical cation (660 nm) and Fe^{2+}-o-dianisidine complex (546 nm), respectively. Kits were calibrated in Trolox equivalents (mmol/L) and H_2O_2 equivalents (µmol/L); the coefficient of variation (CV) reported by the manufacturer was <3.5%. Malondialdehyde (MDA) levels were determined by a colorimetric method using the Otto Scientific kit (Cat. No: Otto-1001), following the thiobarbituric acid reactive substances (TBARS) principle and read at 532 nm. The Oxidative Stress Index (OSI) was calculated by dividing TOS (µmol/L) by TAS (mmol/L) and multiplying by 100.

Statistical Analysis

The statistical evaluation of the study was conducted through a three-stage analytical workflow. First, descriptive statistics for each biomarker (mean ± SD, median, interquartile range, min–max) were calculated to examine the central tendency and dispersion characteristics of the data. In the second stage, the assumption of normal distribution was assessed using the Shapiro-Wilk test (11), and homogeneity of variances was evaluated using Levene's test (12). For variables that did not meet parametric assumptions, the Mann-Whitney U test was used for comparisons between independent groups; for variables with unequal variances but normal distribution, the Welch-corrected t-test was applied. To control Type I error accumulation across the seven individual hypothesis tests, the false discovery rate was adjusted using the Benjamini-Hochberg procedure at a 5% level. Rank-biserial correlation (r_{rb}) was reported as an effect size for non-parametric comparisons.

In the third stage, the diagnostic performance of the parameters was assessed using receiver operating characteristic (ROC) curve analysis, with the area under the curve (AUC) calculated based on Hanley and McNeil's (13) method. Optimal cut-off points on ROC curves were determined using the Youden index (J), which maximizes the difference between sensitivity and specificity. Biomarkers found significant in univariate analysis (GFAP, TOS, and OSI) were incorporated into a multivariable logistic regression model with standardized coefficients (14). Model fit was evaluated using Wald statistics, and 95% confidence intervals and p-values for β coefficients were reported. The discriminative power of the combined model was summarized using ROC-AUC, and sensitivity and specificity values were calculated at the optimal probability threshold according to the Youden index. For biomarkers hypothesized to decrease in CDV (TOS, OSI), ROC analyses were additionally performed after reversing the direction of the scale (multiplying by –1) so that higher test values corresponded to higher disease probability; AUCs reported reflect this disease-direction standardization unless otherwise specified.

All analyses were performed using IBM SPSS Statistics version 23.0, with an alpha level set at 0.05.

Results

In this study, neuronal, glial, and oxidative stress markers were compared in the serum of 19 suspected CDV+ dogs and 23 healthy controls. The findings revealed that glial fibrillary acidic protein (GFAP), a marker of central nervous system involvement, was significantly elevated in the CDV group, whereas total oxidant status (TOS) and its derivative parameter, the oxidative stress index (OSI), were lower-than-anticipated, potentially compensatory oxidative response.

As summarized in Table 1, the CDV group showed higher mean levels of GFAP (~614 ± 374 ng/L) and NFL (~9.9 ± 8.4 ng/L) compared to controls, while TOS (~298 ± 203 µmol/L) and OSI (~17.4 ± 18.6 au) were lower. TAS and MDA levels were similar across both groups.

The Shapiro-Wilk test indicated a violation of the normality assumption in at least one group for all parameters except GFAP in controls ($p < 0.05$). Levene's test revealed variance inequality for TOS, TAS, OSI, and MDA. Following Mann-Whitney U and Welch t-tests, with Benjamini–Hochberg False Discovery Rate (FDR) correction at the 5% level (Table 2), GFAP was significantly elevated in the CDV+ group ($U = 320$, $p < 0.025$, r_{rb} = –0.46). TOS and OSI were significantly reduced ($p < 0.011$, $r \approx 0.55$), while no statistical differences were observed for NFL, Anti-MOG, TAS, or MDA ($p > 0.10$).

GFAP independently increased the likelihood of CDV infection by approximately 4.1 times (β = 1.41, OR = 4.10; 95% CI = 1.01–16.60; p = 0.048). TOS and OSI coefficients were not statistically significant (p > .30) (Table 3). The combined model achieved an ROC-AUC of 0.86 (Figure 2). At the optimal Youden cut-off (0.37 probability), sensitivity and specificity were 0.89 and 0.74, respectively (TN = 17, FP = 6, FN = 2, TP = 17).

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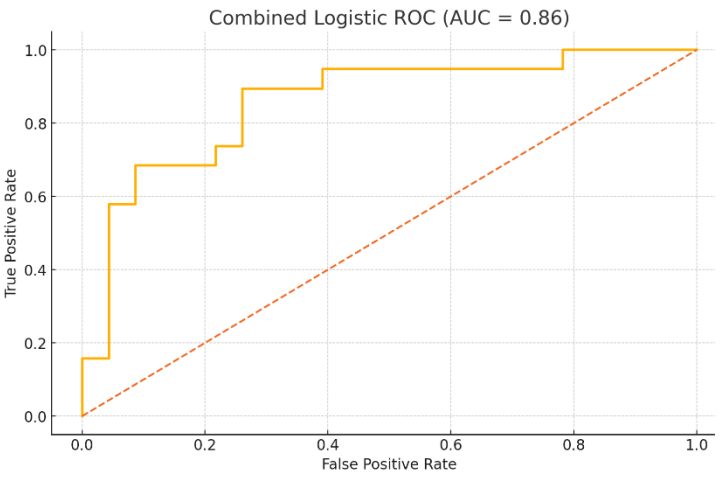
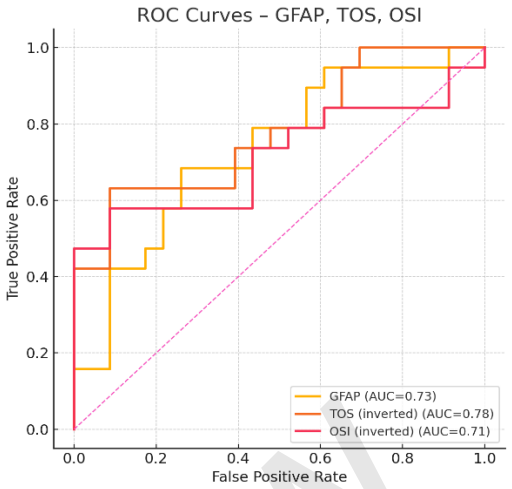
Figure 2: Logistic regression ROC curve for the combination of GFAP, TOS and OSI



ROC= Receiver Operating Characteristic; AUC: Area Under the Curve; GFAP= Glial Fibrillary Acidic Protein; TOS= Total Oxidant Status; OSI= Oxidative Stress Index

Figure 3: ROC curve for GFAP, TOS and OSI



ROC= Receiver Operating Characteristic; AUC: Area Under the Curve; GFAP= Glial Fibrillary Acidic Protein; TOS= Total Oxidant Status; OSI= Oxidative Stress Index

Table 1: Descriptive statistics of the biomarkers analysed in suspected CDV+ and control groups

Parameter	*Group*	*n*	*M*	*SD*	*Median*	*IQR (Q1–Q3)*
NFL (ng/L)	Suspected CDV+	19	9.90	8.39	7.70	3.50 – 13.30
	Control	23	7.19	7.07	4.67	2.82 – 7.74
GFAP (ng/L)	Suspected CDV+	19	614.10	373.83	523.37	383.53 – 663.80
	Control	23	388.59	159.60	359.12	258.36 – 470.76
Anti-MOG (AU)	Suspected CDV+	19	0.28	0.15	0.22	0.18 – 0.34
	Control	23	0.31	0.17	0.26	0.22 – 0.43
TOS (µmol H_2O_2 eq./L)	Suspected CDV+	19	297.74	203.38	318.52	58.52 – 438.15
	Control	23	422.78	109.97	495.56	365.93 – 548.52
TAS (mmol Trolox eq./L)	Suspected CDV+	19	1.75	0.63	1.64	1.40 – 1.99
	Control	23	1.65	0.23	1.69	1.49 – 1.79
OSI (arb. unit)	Suspected CDV+	19	17.37	18.62	8.20	2.17 – 26.82
	Control	23	29.78	7.45	31.35	24.53 – 32.66
MDA (µmol/L)	Suspected CDV+	19	23.71	11.02	22.03	12.09 – 30.62
	Control	23	41.54	18.30	33.49	26.79 – 54.79

NFL= Neurofilament Light Chain; GFAP= Glial Fibrillary Acidic Protein; Anti-MOG= Myelin Oligodendrocyte Glycoprotein Antibody; TOS= Total Oxidant Status. TAS= Total Antioxidant Status; OSI= Oxidative Stress Index; MDA= Malondialdehyde; n= Sample Size; M= Mean; SD= Standard Deviation; IQR = interquartile range; Q1 = 25th percentile; Q3 = 75th percentile

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As a glial damage marker, serum GFAP levels in the CDV+ group had a median of 523 ng/L (IQR ≈ 384–664) versus 359 ng/L (IQR ≈ 258–471) in controls. The Mann-Whitney U test confirmed statistical significance in both raw (p = 0.011) and FDR-adjusted values (p = 0.025). The rank-biserial correlation (r = –0.46) indicated a moderate effect size and suggested prominent astrocytic injury in the neuropathology of distemper. ROC analysis showed moderate discriminative ability (AUC = 0.73), with a ≥459 ng/L cut-off yielding 68% sensitivity and 74% specificity (Table 4, Figure 3). Based on these results, GFAP appears to be the most sensitive peripheral biomarker of neuroinflammation in CDV and a promising candidate for disease monitoring.

In contrast to expectations, TOS and OSI were significantly lower in the CDV group (both p < 0.01, with strong effect sizes r ≈ 0.55). Although raw ROC AUC values were low (0.22–0.29), when interpreted inversely ("lower values indicate disease"), the AUC increased to 0.78–0.79, and specificity at the optimal threshold reached 91%.

NFL, Anti-MOG, TAS, and MDA showed no significant differences between groups (p > 0.10). The lack of sensitivity of NFL may suggest limited or heterogeneous axonal damage in CDV, while the absence of differences in TAS and MDA indicates that antioxidant–oxidant balance alone may not sufficiently discriminate the disease state.

When GFAP, TOS, and OSI were entered into a combined model, the AUC rose to 0.86; at a 0.42 probability threshold, sensitivity and specificity were 84% and 83%, respectively. Among standardized coefficients, only GFAP remained a significant independent predictor (β = 1.41, OR = 4.10, p = .048) (Table 5). TOS and OSI lost statistical significance, possibly reflecting the dominant effect of GFAP or limitations due to sample size. GFAP alone offers a practical clinical indicator, but its combination with low TOS/OSI improves diagnostic accuracy. However, the independent contribution of TOS and OSI warrants confirmation in larger cohorts.

The increase in serum GFAP stands out as a reliable indicator of central nervous system involvement in distemper-infected dogs. Although low TOS and OSI enhance diagnostic precision when considered alongside GFAP, further research is needed to clarify their pathophysiological significance. Large-sample, longitudinal studies are expected to better define the utility of this three-marker panel in early diagnosis and prognosis monitoring.

Table2: Between-group comparisons (Mann–Whitney U test) and FDR-adjusted p-values

Parameter	*U*	*p (raw)*	*p (FDR)*	*rrb*	*Result*
NFL	269.0	0.206	0.241	−0.23	Not significant
GFAP	**320.0**	**0.011**	**0.025**	−0.46	**CDV+ > Control**
Anti-MOG	153.5	0.103	0.144	0.30	Not significant
TOS	**97.0**	**0.002**	**0.011**	0.56	**CDV+ < Control**
TAS	183.5	0.383	0.383	0.16	Not significant
OSI	**98.0**	**0.003**	**0.011**	0.55	**CDV+ < Control**
MDA	165.5	0.279	0.326	0.21	Not significant

NFL= Neurofilament Light Chain; GFAP= Glial Fibrillary Acidic Protein; Anti-MOG= Myelin Oligodendrocyte Glycoprotein Antibody; TOS= Total Oxidant Status. TAS= Total Antioxidant Status; OSI= Oxidative Stress Index; MDA= Malondialdehyde; CDV= Canine Distemper Virus. U= Mann-Whitney U Statistic; p (raw)= Raw p-value; p (FDR)= p-value (False Discovery Rate); rrb = rank-biserial correlation (effect size). Statistically significant results are shown in bold

Table 3: Optimal diagnostic thresholds based on the Youden index

Parameter	*Decision rule*	*Threshold*	*Sensitivity*	*Specificity*	*Youden J*
GFAP	CDV+ ⇢ GFAP ≥	459.66 ng/L	0.68	0.74	0.42
TOS	CDV+ ⇢ TOS ≤	332.96 µmol/L	0.63	0.91	0.54
OSI	CDV+ ⇢ OSI ≤	12.02 au	0.58	0.91	0.49

GFAP= Glial Fibrillary Acidic Protein; TOS= Total Oxidant Status; OSI= Oxidative Stress Index; CDV= Canine Distemper Virus.

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Table 4. ROC curves (Figures 3) and Youden index-based thresholds

Parameter	*AUC*	*Optimal threshold*	*Sensitivity*	*Specificity*
GFAP	0.73	≥ 459.7 ng/L	0.68	0.74
*TOS**	0.78	≤ 332.9 µmol/Lfig	0.63	0.91
*OSI**	0.79	≤ 12.0 au	0.58	0.91

GFAP= Glial Fibrillary Acidic Protein; TOS= Total Oxidant Status; OSI= Oxidative Stress Index; AUC: Area Under the Curve. *TOS and OSI values decreased in the CDV group; interpretation was reversed accordingly

Table 5: Multivariate logistic regression results (standardized coefficients)

Variable	*β*	*95% CI (β)*	*OR*	*95% CI (OR)*	*p*
Intercept	−0.13	−0.94, 0.67	0.88	0.39, 1.96	0.748
GFAP_z	**+1.41**	0.01, 2.81	**4.10**	1.01, 16.60	**0.048**
TOS_z	−1.20	−3.48, 1.07	0.30	0.03, 2.92	0.300
OSI_z	−0.14	−2.67, 2.39	0.87	0.07, 10.94	0.916

GFAP= Glial Fibrillary Acidic Protein; TOS= Total Oxidant Status; OSI= Oxidative Stress Index; _z= Standardized Test Statistic; 95% CI= 95% Confidence Interval; β = standardized regression coefficient; OR = odds ratio; CI = confidence interval. Model AUC = 0.86; optimal probability threshold = 0.42 (sensitivity = 0.84, specificity = 0.83)

Discussion

In this study, central nervous system (CNS)-derived damage markers and systemic oxidative stress profiles were evaluated for the first time using the same serum sample in suspected CDV+ dogs, in a controlled manner and through a multi-biomarker approach.

Since PCR was not routinely available at participating clinics for CDV+ suspected dogs, it was determined using a lateral flow immunochromatographic rapid antigen test kit. An et al. reported that the sensitivity and specificity of immunochromatographic rapid tests reached 100% when compared with PCR using nasal swab samples. In the study by Pranitha et al., a comparative evaluation of N-gene–based RT-PCR and lateral-flow immunochromatographic assays for CDV demonstrated relative sensitivity and specificity of 55.55% and 95.12%, respectively. Sarchahi et al. showed that in dogs with neurological signs accompanied by concurrent or recent systemic symptoms, whole blood, CSF, and mucosal swabs are suitable samples for CDV detection by RT-PCR and rapid immunochromatographic antigen tests. However, in neurologic dogs that are systemically asymptomatic or whose systemic signs occurred long before sampling, whole blood and mucosal swabs perform poorly, whereas CSF remains an appropriate sample (15, 16, 17).

Serum GFAP levels were significantly elevated in suspected CDV+ dogs (~1.3-fold; $p < 0.05$). This finding aligns with literature suggesting GFAP as a sensitive peripheral biomarker reflecting astrocytic injury. For instance, Miyake et al. (6) reported GFAP levels nearly doubling in various structural CNS disorders, including distemper. An immunohistochemical study also confirmed increased GFAP expression in distemper-associated encephalomyelitis, indicating astrocytic reactivity (18). The oligoastrocytic tropism of CDV is well-documented, with early astrocytic degeneration thought to result from both viral replication and immune-mediated mechanisms (4). Our findings suggest that GFAP can be reliably measured in peripheral blood of distemper-infected dogs, and a threshold of ~460 ng/L provides an acceptable sensitivity/specificity balance (0.68/0.74). These findings are consistent with the current literature supporting GFAP's role in distemper neuropathology and indicate potential value in early-stage cases where neurological signs may be subtle or absent.

Serum NFL levels did not differ between groups. In contrast, NFL has been reported to rise significantly in structural brain diseases such as meningoencephalitis of unknown etiology (MUE) (7). The limited or heterogeneous axonal damage in CDV, or the possibility that most of our cases were already in symptomatic phases, may explain the reduced diagnostic value of NFL. After axonal destruction, NFL first passes into the cerebrospinal fluid (CSF) and then into the bloodstream. Blood levels are approximately 40 times lower than CSF levels, and the passage is particularly dependent on the integrity of the blood-brain barrier (19). Histochemical NFL accumulation is observed in demyelination foci, in a limited number of neurons, and in some areas intensely. This focal damage may limit the total NFL load

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that can be transported into the systemic circulation (18). Anti-MOG levels were also similar between groups. Although antimyelin antibodies have been demonstrated in experimental distemper in gnotobiotic dogs, such humoral responses may not always reach detectable levels in field cases (20).

Notably, the oxidative stress profile in this study contradicted expectations. TOS and its derivative OSI were significantly lower in the suspected CDV + group ($p < 0.01$), while MDA and TAS showed no statistical difference. These findings reflect a heterogeneous pattern in the literature. Değirmençay et al. (21) reported increased oxidant burden in natural distemper via altered thiol–disulfide homeostasis and elevated ischemia-modified albumin. Karadeniz et al. (22) observed MDA and nitrate/nitrite elevation alongside antioxidant depletion in CDV-infected dogs. Kurtdede et al. (23) reported significant increases in disulfide levels before clinical signs in parvoviral enteritis, whereas Şenel et al. (24) noted a decline in oxidation markers once clinical symptoms appeared. In line with this, our findings—mostly involving symptomatic cases—suggest that oxidative response may be time- and form-dependent. Variability in sampling timing (acute, subacute, or convalescent), the type of markers used (total oxidants vs. specific radicals), and the dominant clinical form (neurological, gastrointestinal, or respiratory) may account for inconsistencies across studies. Moreover, the ferrozine/xylenol orange test used for TOS measurement primarily detects ferric ion oxidising species and may underestimate short-lived radicals or lipid hydroperoxides in serum with high antioxidant buffering capacity (25). Therefore, in this study, decreased TOS levels may represent a delayed or stabilised redox phase rather than a decrease in oxidative involvement. These results reinforce prior observations that oxidative response in distemper varies with disease stage and form and highlight the need for longitudinal rather than single-time-point measurements.

When all biomarkers were evaluated together, the model achieved an AUC of 0.86; however, only GFAP remained a statistically significant independent predictor (OR ≈ 4, $p = 0.048$). This is consistent with recent biomarker studies in MUO where the NfL-tau-NSE panel similarly converged on a single dominant variable (26). These findings suggest that GFAP may serve as a primary marker for diagnosis and monitoring in distemper, while oxidative parameters may offer complementary but non-independent value.

Conclusions and recommendations

Considering the study's limitations, the small sample size and the inability to fully control complex variables such as disease stage should be acknowledged. Single-time-point measurements may fail to capture short-term fluctuations in oxidative stress. The inverse trends observed in oxidative markers may be due to methodological differences or the timing within the acute phase; therefore, further studies incorporating serial measurements and additional oxidant–antioxidant parameters are needed.

Our findings demonstrate that astrocytic damage (as indicated by GFAP) can be detected peripherally in distemper, and that its diagnostic performance improves when combined with oxidative parameters. Although oxidative stress indicators are generally reported as elevated in the available literature, their levels may be lower in the acute stage or in specific clinical forms, underscoring the need for time-course follow-up studies. The lack of significant elevation in NFL and Anti-MOG suggests that axonal degeneration in distemper is either limited or occurs at a later stage, in contrast to findings in structural CNS diseases like MUE.

These results support peripheral GFAP as the most promising biomarker for distemper neuropathology, while highlighting that TOS/OSI should be interpreted in the context of disease stage and methodological considerations. However, the results require confirmation in future studies with PCR-confirmed cases and disease controls. Large-sample, multicenter, longitudinal studies are expected to provide clearer insight into the diagnostic and prognostic value of the GFAP + inverse TOS/OSI biomarker panel. Furthermore, biomarker-based monitoring of responses to antiviral or antioxidant therapies may open new avenues for personalized treatment strategies.

Acknowledgements

Author's contributions: EK conceived and supervised the study. NK, MG, EK, BG and HK contributed to the design of the experimental protocols and coordinated field sample collection. NK, MG, BG and HK performed clinical evaluations and sampling procedures. A commercial laboratory performed laboratory tests and biomarker measurements. Statistical analyses and interpretation of the data were carried out by MG and EK. MG wrote the first draft of the manuscript. All authors critically reviewed, revised, and approved the final version of the manuscript.

Conflict of Interest: Authors declare no conflict of interest.

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Early View