Received: 16 June 2021 | Accepted: 10 August 2021

DOI: 10.1111/jvim.16251

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Serum phosphorylated neurofilament heavy chain as a diagnostic biomarker for progressive myelomalacia in dogs with thoracolumbar intervertebral disc herniation

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Funding information
Center for Companion Animal Health, School of Veterinary Medicine, UC Davis, Grant/Award Number: 2016-48-R

Abstract

Background: Serum phosphorylated neurofilament-heavy chain (pNF-H) has not been longitudinally evaluated in dogs that develop progressive myelomalacia (PMM) after Type I intervertebral disc herniation (IVDH).

Objectives: To determine if serum pNF-H concentrations would predict outcome of neuroligical disease in dogs with acute, severe thoracolumbar myelopathy secondary to Type I IVDH.

Animals: Thirty-nine client-owned dogs with thoracolumbar myelopathy secondary to IVDH.

Methods: Prospective controlled cohort study. Serum was collected from dogs undergoing hemilaminectomy at multiple timepoints. Final neurological status was established at 12 months and groups were stratified accordingly. Comparisons between outcome and pNF-H concentration at each timepoint was examined using Kruskal-Wallis analysis of variance on ranks and receiver operator characteristics curve analysis.

Results: Median serum pNF-H concentrations were not significantly different between deep pain negative dogs that did or did not recover at any timepoint (baseline: 0.37 ng/mL [0-0.9 ng/mL] vs 0 ng/mL [0-0.9 ng/mL], $P > 1$; 24 hours: 1.25 ng/mL [0.35-7.23 ng/mL] vs 1.53 ng/mL [0-11.94 ng/mL], $P > 1$; 48 hours: 1.22 ng/mL [0.63-6.62 ng/mL] vs 2.12 ng/mL [0-20.72 ng/mL], $P > 1$; 72 hours: 2.77 ng/mL [1.33-6.62 ng/mL] vs 16.69 ng/mL [4.02-40.12 ng/mL], $P > 1$). Dogs that developed PMM had significantly higher serum pNF-H concentrations after surgery compared to all other cohorts at 24 hours: 39.88 ng/mL (25.74-50.68 ng/mL); $P < .05$ and 72 hours: 223.9 ng/mL (155.4-263.7 ng/mL); $P < .05$. A serum pNF-H concentration

Abbreviations: DPN, deep pain negative; DPN-NR, deep pain negative—nonrecovery; DPN-PMM, deep pain negative—progressive myelomalacia; DPN-R, deep pain negative—recovery; DPP, deep pain positive; IQR, interquartile range; IVDH, intervertebral disc herniation; NSAID, nonsteroidal anti-inflammatory drug; PMM, progressive myelomalacia; pNF-H, phosphorylated neurofilament-heavy chain; SCI, spinal cord injury.

≥31.39 ng/mL was 83.33% sensitive and 100% specific for identifying PMM in this cohort.

Conclusions and Clinical Importance: Serum pNF-H is a promising biomarker for antemortem diagnosis of PMM in dogs with acute, severe thoracolumbar myelopathy secondary to Type I IVDH.

KEYWORDS
canine, chondrodystrophy, myelopathy, prognosis, spinal cord injury

1 | INTRODUCTION

Thoracolumbar intervertebral disc herniation (IVDH) is a common cause of spinal cord injury (SCI) in dogs.[1,2] While IVDH can cause a variety of clinical signs, the most severe cases suffer from a functionally complete SCI, resulting in paraplegia with loss of nociception.[3] To date, the absence of nociception is the single most useful prognostic indicator for functional recovery.[1,3-5] While approximately 42% to 62% of dogs with loss of nociception will recover ambulation and continence after surgical intervention, 11% to 33% of such dogs will develop progressive myelomalacia (PMM) or ascending/descending hemorrhagic myelomalacia.[1,3,5-10] Currently, there is no established antemortem biomarker to distinguish between dogs that will regain function, remain paraplegic, or develop PMM, exposing a critical barrier for informed case management.

Identification of a minimally invasive biomarker that can predict outcome after functionally complete SCI secondary to IVDH would inform owner expectations and facilitate financial and ethical decision-making. Multiple biomarker candidates have been evaluated in dogs with IVDH, including serum concentrations of glial fibrillary acidic protein, S100β, creatine kinase, lactate, and myelin basic protein.[11-15] However, none have sufficient prognostic value. Importantly, no candidate biomarker has been followed longitudinally in PMM cases.[11-16] Phosphorylated neurofilament-heavy chain (pNF-H) has variable success as a predictive biomarker for functional recovery across several species after acute severe SCI.[13-15,17-20] Specifically, the predictive value of serum pNF-H concentration of identifying dogs that recover vs dogs that do not after IVDH has been inconsistent across studies, which might be a reflection of the variability in the time of evaluation relative to injury.[13-15] Moreover, while previous studies have commented that dogs with PMM have elevated serum pNF-H concentrations relative to other study cohorts, this particular study sample has neither been specifically evaluated as an individual study cohort, nor serially monitored in the acute postoperative period.[13-15]

Therefore, the purpose of this study was to evaluate serum pNF-H concentrations within 24 hours of initial injury and longitudinally thereafter in dogs with functionally complete SCI secondary to IVDH. We hypothesized that (a) serum pNF-H concentration would be associated with clinical outcome, with the highest pNF-H concentrations in nonrecovering dogs; and (b) serum pNF-H concentration would increase over time in dogs with PMM.

2 | MATERIALS AND METHODS

2.1 | Case selection

A prospective controlled cohort study was performed. Client owned dogs presenting to the University of California, Davis (UC Davis) William R. Pritchard Veterinary Medical Teaching Hospital with a thoracolumbar myelopathy, with and without nociception, secondary to Hansen Type 1 IVDH, were prospectively enrolled from January 2017 to March 2019. All samples were collected with informed client consent and the study was approved by the UC Davis Institutional Animal Care and Use Committee (protocol # 19648) and the UC Davis Veterinary Medical Teaching Hospital Institutional Clinical Trials Review Board.

All cases that met the following criteria were included in this study: dogs with thoracolumbar IVDH resulting in SCI; onset of nonambulatory status, if present, ≤24 hours before hospital admission; diagnosis of IVDH confirmed on either magnetic resonance imaging or computed tomography scan, followed by subsequent surgical decompression within 24 hours of presentation. Dogs with concurrent neurological disease or previous SCI that underwent surgery were excluded from the study. However, dogs with successful medical management of a presumed previous SCI that recovered from all clinical signs at least 3 months before hospital admission were not excluded.

Neurological status was graded using the Modified Frankel Scale (MFS) as follows: 5: Apparent pain with no neurological deficits; 4: Ambulatory paraparesis; 3: Nonambulatory paraparesis; 2: Paraplegia, intact superficial nociception; 1: Paraplegia, intact deep nociception, absent superficial nociception; 0: Paraplegia, absent deep nociception.[21-23] Dogs were divided into study cohorts based on the presence or absence of deep nociception at presentation. Absent nociception (deep pain negative [DPN]) was defined as a lack of conscious response to pinching over the bone of the medial and lateral digits of the pelvic limbs and coccygeal vertebrae using hemostats. Dogs with an MFS score of ≥1 were pooled into a single group (deep pain positive; DPP). Dogs were followed prospectively as detailed below and categorized by final outcome during data analysis. Dog signalment, weight, administration of anti-inflammatory drugs before presentation, duration of loss of ambulation before presentation, as well as length of hemilaminectomy, were recorded for all cases. Durotomy was not performed on any case included in this study.

2.2 | Clinical outcome assessment

Neurological status was evaluated and scored according to the MFS at presentation and every 12 hours postoperatively by a board-certified neurologist or neurology resident throughout hospitalization for all dogs. After hospital discharge, neurological status was evaluated by neurological examination at 10 to 14 days postoperatively, as well as at 6 to 8 weeks postoperatively. Owners of dogs that survived to discharge were contacted a minimum of 1 year after surgery to confirm final neurological outcome. Dogs that were suspected to have developed PMM were identified by progressive loss of muscle tone and segmental spinal reflexes cranial and caudal to the known lesion site over serial neurological examinations. These dogs were humanely euthanized after joint discussion between the clinician and pet owner. PMM was confirmed in 2 cases via histopathologic examination.

Dogs were assigned to a functional outcome status at the time of data analysis based on the following criteria: recovery was defined as a lack of apparent thoracolumbar paraspinal hyperesthesia, urinary and fecal continence, and an ability to ambulate at least 10 consecutive steps unassisted. Dogs that did not meet these criteria were considered nonresponders (nonrecovery). For data analysis, dogs were grouped by outcome: dogs with intact nociception at presentation that were responders (DPP), DPN dogs that recovered (DPN-R), DPN dogs that did not recover (DPN-NR), and DPN dogs that did not recover and developed PMM that were subsequently euthanized (DPN-PMM; Figure 1).

2.3 | Sample collection and testing

Blood was collected into a serum separator tube immediately before surgery (baseline; within 24 hours of presumed initial injury), 24, 48, and 72 hours postoperatively. Thus, all timepoints were within 96 hours of presumed initial injury. After clot formation, tubes were centrifuged, the serum was aspirated and then stored at −80°C until analysis.

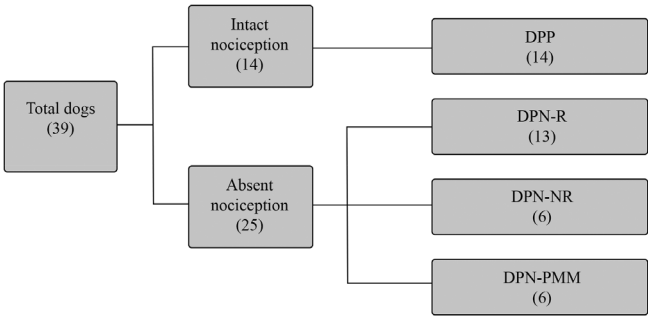

FIGURE 1 Distribution of enrolled dogs within each outcome group. DPP, deep pain positive—recovery; DPN-R, deep pain negative—recovery; DPN-NR, deep pain negative—nonrecovery; DPN-PMM, deep pain negative—progressive myelomalacia

Serum p-NF-H concentrations were measured using a commercially available ELISA kit (Cat# ELISA-pNF-H-V2, from EnCor Biotechnology Inc, Gainesville, Florida) following the manufacturer's instructions. Wash cycles within the assay were performed via BioRad 1575 Immuno-Wash Microplate Washer (BioRad Laboratories, Hercules, California). Standards and samples were evaluated in triplicate. To maintain optical densities within the linear range of the standard curve, samples were diluted 1 : 2, 1 : 25, or 1 : 50. Absorbance was read at 450 nm using an 800 TS Absorbance Microplate reader (BioTek, Winooski, Vermont). For quality control, intra- and interassay coefficients of variability (CV) were calculated. Intra-assay CV was calculated from the optical densities of 3 randomly selected samples per plate from 5 independent experiments. Interassay CV was calculated from pNF-H concentration of 3 samples tested across 3 independent experiments.

2.4 | Statistical analysis

Serum pNF-H concentrations at each timepoint were stratified by outcome group and tested for normality using the Shapiro-Wilk test.

TABLE 1 Dog clinical data

	Deep pain sensation	
	Present	Absent
	n = 14	n = 25
Sex		
Male	7 (50%)	11 (44%)
Female	7 (50%)	14 (56%)
Breed		
Dachshund	11 (78%)	6 (24%)
Chihuahua	1 (7%)	4 (16%)
Other	2 (14%)	15 (60%)
Modified Frankel Score		
Grade 0		25 (100%)
Grade 1	2 (14%)	–
Grade 2	3 (21%)	–
Grade 3	7 (50%)	–
Grade 4	2 (14%)	–
Grade 5	0 (0%)	–
Prior medical treatment		
None	5 (35%)	11 (44%)
Nonsteroidal anti-inflammatory	9 (64%)	13 (52%)
Corticosteroid	0 (0%)	1 (4%)
Length of hemilaminectomy (# disc spaces)		
1	8 (57%)	9 (36%)
2	5 (35%)	6 (24%)
3	1 (7%)	8 (32%)
4	0 (0%)	2 (8%)

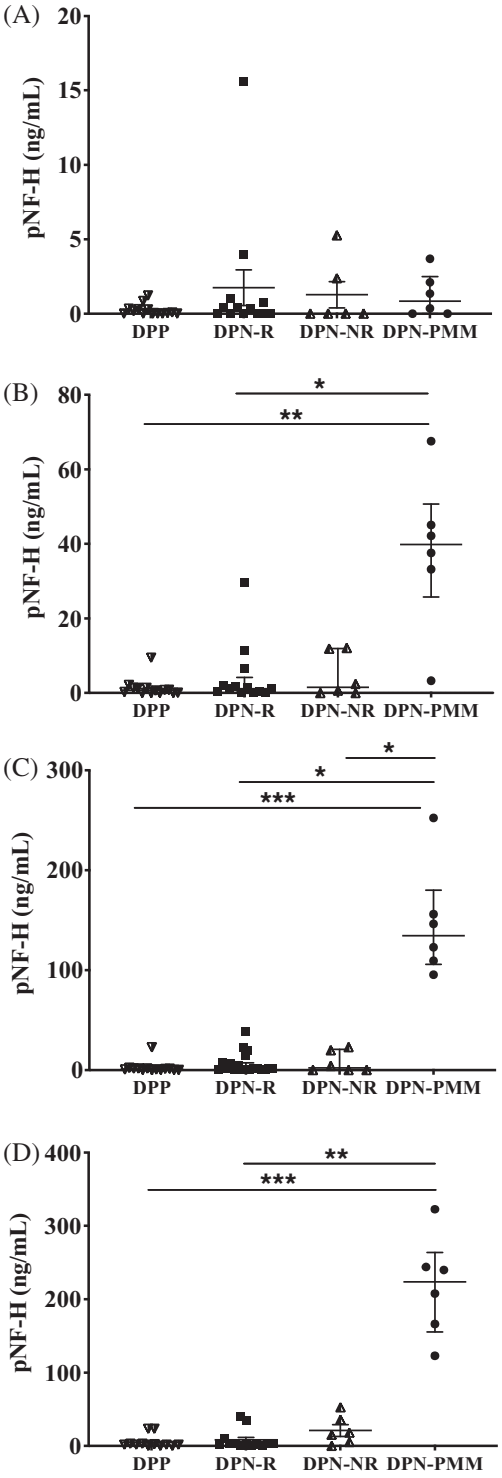

FIGURE 2 Serum phosphorylated neurofilament-heavy (pNF-H) concentrations were increased in deep pain negative—progressive myelomalacia (DPN-PMM) dogs at all postoperative timepoints. A, Serum pNF-H concentrations were not significantly different across deep pain positive (DPP; n = 14), deep pain negative—recovery (DPN-R; n = 13), deep pain negative—nonrecovery (DP-NR; n = 6), and DPN-PMM groups at baseline. B, DPN-PMM dogs had significantly increased serum pNF-H concentrations compared to DPP (**P < .01) and DPN-R (*P < .05) dogs at 24 hours postoperatively. C, DPN-PMM dogs had significantly increased serum pNF-H concentrations compared to DPP (***P < .001), DPN-R (*P < .05), and DPN-NR (*P < .05) dogs at 48 hours postoperatively. D, DPN-PMM dogs had increased serum pNF-H concentrations compared to DPP (***P < .001) and DPN-R (**P < .01) dogs at 72 hours postoperatively. Comparisons based on a Kruskal-Wallis analysis of variance on ranks with post hoc Dunn's method; bars represent group median and interquartile range

Comparisons across study groups were analyzed by Kruskal-Wallis analysis of variance (ANOVA) on ranks with post hoc multivariate analyses and presented as the median with interquartile range. A separate longitudinal analysis of pNF-H serum concentrations of DPN-NR and PMM cohorts were analyzed by 2-way repeated measures ANOVA with Sidak's multiple comparisons test. Based on initial data analysis, receiver-operating characteristics (ROC) curve analysis was performed to determine the accuracy of serum pNF-H concentration to predict PMM at 24 h and 48 hours.

Associations between baseline pNF-H concentrations and (a) age or (b) body weight were evaluated using simple linear regression. Associations between baseline pNF-H concentrations and sex, duration of nonambulation and baseline nonsteroidal anti-inflammatory drug (NSAID) status were evaluated using a Mann-Whitney U test. Associations between baseline pNF-H concentration, length of hemilaminectomy, and outcome group were estimated using Kruskal-Wallis ANOVA tests. Effect of anti-inflammatory drug administration on outcome was evaluated by a Fisher's exact test.

Intra-assay coefficient of variability was calculated for each sample within an experiment by dividing the SD of the optical densities per plate (run in triplicate) by the mean of the 3 optical densities. The CV for all samples across 5 experiments were averaged for a final intra-assay CV. Interassay coefficient of variability was calculated for each sample by dividing the SD of calculated concentrations of each experiment by the mean pNF-H concentration across 3 independent experiments. The CV for all samples across 3 experiments were averaged for a final interassay CV. All analyses and graphics were made using statistical software (GraphPad Prism 2021; StataCorp 2015), and P < .05 was considered statistically significant.

3 | RESULTS

3.1 | Clinical features

A total of 39 dogs were enrolled. The Dachshund was the most common breed encountered (n = 17), with 5 Chihuahuas, 3 French bulldogs, 2 Maltese, 2 Beagles, 2 mixed breed dogs and 1 each of Pembroke Welsh Corgi, Whippet, Pekinese, Cocker spaniel, Toy poodle, Jack Russell terrier and "Pitbull" terrier. Sexes were similarly represented (46% male dogs and 54% female; Table 1). All nonambulatory dogs presented within 24 hours of loss of ambulation. Fourteen dogs were DPP with a MFS of: 4 (n = 2), 3 (n = 7), 2 (n = 3), and 1 (n = 2). All 14

TABLE 2 Serum pNF-H concentrations (ng/mL) for each cohort of dogs over time

	Deep pain positive	Deep pain negative		
	DPP	DPN-R	DPN-NR	DPN-PMM
	n = 14	n = 13	n = 6	n = 6
Baseline	0.07 (0-0.32)	0.37 (0-0.9)	0 (0-3.1)	0.86 (0-2.51)
	(*P* > 1)	(*P* > 1)	(*P* > 1)	
24 h	0.72 (0.01-1.4)	1.25 (0.35-7.23)	1.53 (0-11.94)	39.88 (25.74-50.68)
	(*P* = .001)†	(*P* = .02)†	(*P* = .07)	
48 h	0.96 (0.06-1.68)	1.22 (0.63-6.62)	2.12 (0-20.72)	134.50 (105.9-18)
	(*P* = .0007)†	(*P* = .02)†	(*P* = .01)†	
72 h	1.86 (0.59-3.07)	2.77 (1.33-6.62)†	16.69 (4.02-40.12)	223.90 (155.4-263.7)
	(*P* = .0004)†	(*P* = .004)	(*P* = .27)	

Note: Data are presented as median and interquartile range.
Abbreviations: DPP, deep pain positive (controls); DPN-R, deep pain negative—recovery; DPN-NR = deep pain negative—nonrecovery; DPN-PMM, deep pain negative—progressive myelomalacia.
†Significant difference (*P* < .05) from pNF-H concentration of DPN-PMM group at the same time point.

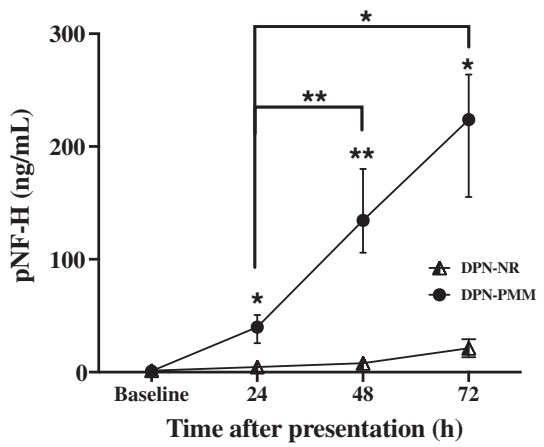

FIGURE 3 Phosphorylated neurofilament-heavy (pNF-H) serum concentrations progressively increased in deep pain negative—progressive myelomalacia (DPN-PMM) dogs in the acute postoperative period. Longitudinal evaluation demonstrated increased serum pNF-H concentration from baseline at 24 hours (*P < .05), 48 hours (**P < .01), and 72 hours (*P < .05) postoperatively in DPN-PMM dogs (n = 6). Moreover, compared to the 24-hour timepoint, serum pNF-H in DPN-PMM dogs was increased at 48 hours (**P < .01) and 72 hours (*P < .05). Comparisons based on a 2-way repeated measures analysis of variance with post hoc Sidak's multiple comparison test; bars represent group median and interquartile range

DPP dogs met recovery criteria. Twenty-five dogs were categorized as DPN (MFS: 0). Of these 25 dogs, 13 dogs recovered (DPN-R), 6 did not recover (DPN-NR), and 6 dogs developed PMM (DPN-PMM; Figure 1). Among dogs of all groups, compression of the spinal cord spanned 1 to 4 intervertebral disc spaces (Table 1). Length of compression (and subsequent hemilaminectomy) was not associated with outcome (*P* = .09). Before presentation, 58% of dogs received NSAID (n = 22) or anti-inflammatory corticosteroid (n = 1) medication (Table 1). Postoperatively, 74% of dogs received NSAID (n = 24) or anti-inflammatory corticosteroid (n = 5) medication. Administration of anti-inflammatory medication at any timepoint was not associated with outcome (*P* = .35).

All DPP, DPN-R, and DPN-NR dogs survived to discharge. DPN-PMM dogs (n = 6) were humanely euthanized between 24 and 72 hours after the onset of progressive clinical signs suggestive of PMM. One of the 6 dogs lacked a cutaneous trunci reflex at presentation, with other progressive signs suggestive of PMM noted upon recovery from anesthesia after surgery. The remaining 5 dogs began to exhibit PMM signs 24 to 48 hours postoperatively.

3.2 | Assay variability

The average intra-assay CV was 4.7%. The average interassay CV was 5.7%.

3.3 | Baseline serum pNF-H concentrations

Median baseline serum pNF-H concentrations were similar across all groups (DPP: 0.07 ng/mL [interquartile range (IQR) 0-0.32 ng/mL]; DPN-R: 0.37 ng/mL [IQR 0-0.9]; DPN-NR: 0 ng/mL [IQR 0-3.1], and DPN-PMM: 0.86 ng/mL [IQR 0-2.51]; *P* = .5). Median baseline serum pNF-H concentration for all dogs (0.09 ng/mL [IQR 0-0.86 ng/mL]) was not significantly associated with age (*P* = .85), body weight (*P* = .72), sex (*P* = .85), length of hemilaminectomy (*P* = .84), preoperative administration of anti-inflammatory drugs (*P* = .7), or duration of loss of nonambulation (*P* = .75).

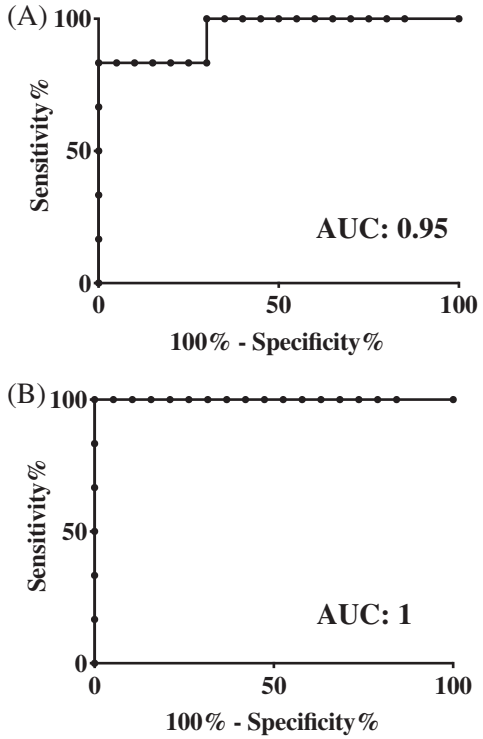

FIGURE 4 Serum phosphorylated neurofilament-heavy (pNF-H) might be a specific antemortem disease marker for progressive myelomalacia. A, Receiver-operating characteristics (ROC) curve comparing serum pNF-H concentration of deep pain negative—progressive myelomalacia (DPN-PMM) dogs (n = 6) to deep pain positive (DPP; n = 14), deep pain negative—recovery (DPN-R; n = 13), and deep pain negative—nonrecovery (DP-NR; n = 6) dogs at 24 hours postoperative. Area under the curve (AUC) was 0.95; serum pNF-H > 31.39 ng/mL was 83.33% sensitive and 100% specific for identification of PMM. B, ROC curve comparing serum pNF-H concentration of DPN-PMM dogs to DPP, DPN-R, and DP-NR dogs at 48 hours postoperative. Area under the curve (AUC) was 1; serum pNF-H ≥ 67.1 was 100% sensitive and 100% specific for identification of PMM

3.4 | Postoperative serum pNF-H concentrations

The length of hemilaminectomy was not associated with pNF-H concentration at 24 hours ($P = .88$), 48 hours ($P = .61$), nor 72 hours ($P = .32$) postsurgery. At 24 hours, median serum pNF-H concentration in the DPN-PMM group (39.88 ng/mL [IQR 25.74-50.68 ng/mL]) was significantly higher than DPP (0.72 ng/mL [IQR 0.01-1.40 ng/mL]; $P = .001$) and DPN-R groups (1.25 ng/mL [IQR 0.35-7.23 ng/mL]; $P = .02$), but not the DPN-NR group (1.53 ng/mL [IQR 0-11.94 ng/mL]; $P = .07$; Figure 2A; Table 2). At 48 hours, median serum pNF-H concentration in the DPN-PMM group (134.5 ng/mL [IQR 105.9-180 ng/mL]) was significantly higher than the DPP (0.96 ng/mL [IQR 0.06-1.68 ng/mL]; $P = .0007$), DPN-R (1.22 ng/mL [IQR 0.63-6.62 ng/mL]; $P = .02$) and DPN-NR groups (2.12 ng/mL [IQR 0.00-20.72 ng/mL]; $P = .01$; Figure 2B; Table 2). At 72 hours, median serum pNF-H concentration in the DPN-PMM group (223.9 ng/mL [IQR 155.4-263.7 ng/mL]) was significantly higher than the DPP (1.86 ng/mL [IQR 0.59-3.07 ng/mL]; $P = .0004$) and DPN-R groups (2.77 ng/mL [IQR 1.33-6.62 ng/mL]; $P = .004$) but not the DPN-NR group (16.69 ng/mL [IQR 4.02-40.12 ng/mL]; $P = .27$; Figure 2C; Table 2). There were no significant differences between any other pair of groups at 24, 48, or 72 hours postoperatively.

Repeated measure analysis of DPN-NR and DPN-PMM groups revealed a progressive increase in median serum pNF-H concentrations in DPN-PMM dogs. Compared to baseline, DPN-PMM serum pNF-H concentrations were increased at 24 hours ($P = .03$), 48 hours ($P = .006$), and 72 hours ($P = .03$; Figure 3). Notably, serum pNF-H concentrations were also increased at 48 hours ($P = .006$) and 72 hours ($P = .04$) when compared to the 24-hour timepoint (Figure 3). Conversely, serum pNF-H concentration were not significantly increased over time in DPN-NR dogs. Compared to baseline, neither 24 hours ($P = .72$), 48 hours ($P = .75$), nor 72 hours ($P = .3$) were significantly elevated.

Receiver-operating characteristics curve analysis at 24 hours postoperatively demonstrated a calculated area under the curve (AUC) of 0.95 (CI: 0.85-1; $P = .001$; Figure 4A). A serum pNF-H concentration ≥31.39 ng/mL yielded the optimal discrimination between the DPN-PMM group and all other groups, with a sensitivity of 83.33% (CI: 35.9%-99.6%) and specificity of 100% (CI: 82.4%-100%). At 48 hours postoperatively, the calculated AUC was 1 (CI: 1-1; $P = .0003$; Figure 4B). A serum pNF-H concentration ≥67.1 was 100% sensitive (CI: 54.07%-100%) and 100% specific (CI: 83.16%-100%) for PMM. Serum pNF-H concentrations greater than 31 ng/mL preceded clinical signs of PMM in 67% of DPN-PMM dogs.

4 | DISCUSSION

This study examined the temporal relationship of serum pNF-H concentrations and functional recovery in dogs with complete SCI secondary to IVDH. We found that serum pNF-H concentrations, before and after surgical decompression (up to 72 hours), were not associated with recovery in DPN dogs. However, our data indicated that serum pNF-H concentration might accurately identify dogs with PMM by 24 hours after surgery. In fact, we detected elevated serum pNF-H at the same time as or preceding clinical signs of PMM in 5/6 cases, further suggesting the value of this test in this cohort of dogs.

Neurofilaments are abundant cytoskeletal proteins of axons. The severe and progressive axonal degeneration in PMM, coupled with the stability and immunogenicity of pNF-H in biological fluid, is mirrored by the progressive increase in serum pNF-H concentration observed in PMM cases presented here.[24-26] An objective, noninvasive tool to detect PMM cases antemortem would aid in clinical management of these cases and may reduce financial and emotional burden of the pet owner.

All DPN-PMM dogs presented within 24 hours of presumed initial injury and were taken to surgery as soon as possible after admission. Thus, serum pNF-H concentrations are closely representative of the first 96 hours after initial injury. Longitudinal evaluation of serum pNF-H concentrations in PMM dogs revealed a marked, progressive increase after initial injury. However, we were not able to

detect a difference in serum pNF-H across groups at baseline, representing the initial 24 hours after injury. While these findings are consistent with a previous study,[15] they are in contrast to earlier reports that found a correlation between serum pNF-H concentration at the time of hospital admission and functional outcome.[13,14] In particular, serum pNF-H concentrations at the time of admission observed in these studies ranged from 0 to 60.4 ng/mL in PMM cases, which is markedly higher than values obtained in this study (0-2.51 ng/mL). This apparent discrepancy likely reflects the difference in time from presumptive SCI to hospital admission across studies. Given the temporal dynamics of serum pNF-H within the first 72 hours after acute SCI observed in rodent models, we limited case selection in this study to dogs that presented within 24 hours of presumptive SCI.[19] In comparison, previous studies included dogs 72 to 96 hours after SCI.[13-15] Thus, the majority of timepoints evaluated in this present study were within the preadmission timeframe of all other studies. Therefore, timing of sampling relative to time of presumptive SCI likely accounts for the variability in results across studies in dogs with IVDH and will be an important consideration in interpretation of results of future studies.

The time course of serum pNF-H concentrations after functionally complete SCI in dogs secondary to IVDH is described.[15] Progressively increased serum pNF-H concentrations are observed in the first 14 days after SCI in all dogs.[15] However, dogs that do not recover had a greater increase in serum pNF-H concentration between postoperative days 3 and 14.[15] However, our analysis only extended to 96 hours after SCI, whereby serum pNF-H concentrations were the highest observed. Thus, we were not able to detect a time of peak pNF-H concentration in this study sample.

The increase in serum pNF-H concentrations in PMM dogs observed in this study provides rationale for further investigation and validation of the diagnostic accuracy of this test in dogs with this disease. However, an absolute serum concentration of pNF-H might not appropriately discriminate PMM-affected dogs because of technical variability over time. For example, the reported serum pNF-H concentration of PMM dogs in this study were markedly higher than reported values using the same assay.[15] The intra- and interassay CV for this present study was appropriately low, suggesting high reproducibility of this assay. However, our laboratory has extensive experience measuring pNF-H concentration in biological fluid using this assay and we have found that the absolute pNF-H concentration might fluctuate across assays with time. Importantly, we have observed that the magnitude of change in pNF-H concentration and relationship to control samples remains constant. Within our PMM group, serum pNF-H concentrations were increased 46-fold by 24 hours from those at baseline. Thus, evaluating the magnitude of change in test samples, rather than an absolute value, might allow for necessary flexibility of the clinical environment, given the variability in time of presentation to the hospital and lack of precision in identifying the exact time of SCI.

This study was limited by a small sample size and should be replicated in a larger cohort of dogs. These data provide the rationale for a multi-institutional clinical trial to longitudinally evaluate serum pNF-H in dogs after acute SCI secondary to IVDH. In particular, longitudinal evaluation of serum pNF-H in PMM cases relative to the onset of clinical signs of PMM should be emphasized. An additional limitation was a lack of confirmed diagnosis of PMM in all cases. Given the potential clinical importance of serum pNF-H concentrations as an antemortem diagnostic biomarker for this clinical sample, a confirmed diagnosis is necessary for future studies to ensure appropriate validation.

ACKNOWLEDGMENT

Funding provided by Center for Companion Animal Health, School of Veterinary Medicine, UC Davis, 2016-48-R. This work was presented in part at the 2019 ACVIM Forum, Phoenix, AZ. We acknowledge Daniel York, PhD, for his experimental assistance and the veterinary technicians of the Neurology/Neurosurgery service at UC Davis for their invaluable assistance with sample collection.

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

All samples were collected with informed client consent and the study was approved by the University of California, Davis IACUC (protocol # 19648) and the University of California, Davis Veterinary Medical Teaching Hospital Institutional Clinical Trials Review Board.

HUMAN ETHICS APPROVAL DECLARATION

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

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How to cite this article: Murthy VD, Li C-F, Hicks J, et al. Serum phosphorylated neurofilament heavy chain as a diagnostic biomarker for progressive myelomalacia in dogs with thoracolumbar intervertebral disc herniation. *J Vet Intern Med*. 2021;35(5):2366-2373. doi:10.1111/jvim.16251