AJVR

AVMA

Neurofilament light chain concentration does not correlate with disease status in Labrador Retrievers affected with idiopathic laryngeal paralysis

Jacquelyn H. Perino, DVM, MS[1]; Margaret Patterson, BS[1]; Mehdi Momen, PhD[1]; Mina Borisova, BS[2,3]; Amanda Heslegrave, PhD[2,3,4,5]; Henrik Zetterberg, MD, PhD[2,3,4,5]; Jordan Gruel, BS[1]; Emily E. Binversie, DVM, PhD[1]; Ryan S. Anderson, BS[1]; Helena Rylander, DVM, DACVIM[6]; Starr Cameron, BVetMed, MS, DACVIM[6]; Benjamin Miranda, DVM[1]; Gabi Conidi[1]; John Svaren, PhD[7]; Susannah J. Sample, DVM, PhD, DACVS[1]*

[1]Comparative Genetic Research Laboratory, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI
[2]Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK
[3]UK Dementia Research Institute, University College of London, London, UK
[4]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Mölndal, Sweden
[5]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
[6]Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI
[7]Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI

*Corresponding author: Dr. Sample (susannah.sample@wisc.edu)

OBJECTIVE
The aim of this study was to investigate whether plasma neurofilament light chain (pNfL) concentration was altered in Labrador Retrievers with idiopathic laryngeal paralysis (ILP) compared to a control population. A secondary aim was to investigate relationships between age, height, weight, and body mass index in the populations studied.

ANIMALS
123 dogs: 62 purebred Labrador Retrievers with ILP (ILP Cases) and 61 age-matched healthy medium- to large-breed dogs (Controls).

METHODS
Dogs, recruited from August 1, 2016, to March 1, 2022, were categorized as case or control based on a combination of physical exam, neurologic exam, and history. Blood plasma was collected, and pNfL concentration was measured. pNfL concentrations were compared between ILP Cases and Controls. Covariables including age, height, and weight were collected. Relationships between pNfL and covariables were analyzed within and between groups. In dogs where 2 plasma samples were available from differing time points, pNfL concentrations were measured to evaluate alterations over time.

RESULTS
No significant difference in pNfL concentration was found between ILP Cases and Control (P = .36). pNfL concentrations had moderate negative correlations with weight and height in the Control group; other variables did not correlate with pNfL concentrations in ILP Case or Control groups. pNfL concentrations do not correlate with ILP disease status or duration in Labrador Retrievers.

CLINICAL RELEVANCE
There is no evidence that pNfL levels are altered due to ILP disease duration or progression when compared with healthy controls. When evaluating pNfL concentrations in the dog, weight and height should be considered.

Keywords: neurofilament light chain, dog, neuropathy, peripheral neuropathy, axonopathy

Canine idiopathic laryngeal paralysis (ILP) is a common degenerative peripheral polyneuropathy in dogs.[1,2] ILP is considered an axonopathy, characterized by degeneration and loss of the largest and longest axons, with the recurrent laryngeal nerve and the sciatic nerve showing the most substantial clinical consequences.[2–4] Dysfunction in peripheral nerves leads to a variety of clinical signs including respiratory distress, stridor, coughing, gagging,

Received December 18, 2023
Accepted January 25, 2024

doi.org/10.2460/ajvr.23.12.0292

dysphagia, regurgitation, loss of pelvic limb proprioception, and progressive paraparesis.[2,5,6] ILP primarily affects older (> 9 years) large- to giant-breed dogs.[6] Although ILP is documented in many breeds, approximately 70% of cases are seen in Labrador Retrievers.[2,6,7] ILP shares many pathophysiologic, histopathologic, and clinical features with inherited peripheral neuropathies seen in humans, including Charcot-Marie-Tooth (CMT) disease type 2 and distal hereditary motor neuropathy, making it a promising spontaneous large animal disease model candidate for inherited peripheral neuropathy.

Neurofilament light chain (NfL) concentration can be used as a sign of axonal degeneration and in humans is a promising potential biomarker for a wide host of neurodegenerative diseases.[8] NfL is 1 of 4 subunits, along with neurofilament middle chain, neurofilament heavy chain, and α-internexin, that comprise heteropolymer neurofilament proteins forming the neuronal cytoskeleton.[9] All 4 subunits act together to aid in the growth of axonal diameter and act as axonal scaffolding.[9] NfL has proven to be stable, soluble, and abundant in both CSF and plasma.[9,10] Although NfL in the CSF and blood increases during the normal aging process in people, higher levels are found in several human neurodegenerative diseases.[11]

Currently, NfL is used to aid in diagnosis, inform prognosis, and monitor treatment response for a variety of human neurodegenerative diseases.[11–13] The potential to use NfL to track disease progression would allow for more robust clinical trials and treatment response monitoring.[14] In dogs, plasma neurofilament light chain (pNfL) can be measured effectively and has been shown to increase in dogs affected with conditions that affect the CNS.[15,16] It is currently not known whether pNfL is of clinical utility for any peripheral neuropathy in dogs.

The aim of this study was to investigate whether pNfL concentration was altered in Labrador Retrievers affected by ILP compared to an aged control population. A secondary aim was to investigate the relationships between pNfL, age, height, weight, and body mass index (BMI) in the populations studied. Our hypothesis was that Labrador Retrievers affected with ILP would have significantly higher pNfL concentration when compared with an age-matched control population comprised of medium- to large-breed dogs. Our secondary hypothesis was that in the populations studied, comprised of aged dogs, there would be no correlation between age, height, weight, or BMI and pNfL concentration.

Methods

Dogs were recruited from the UW Veterinary Care Hospital at the University of Wisconsin-Madison from August 1, 2016, to March 1, 2022. All data collection was conducted with the approval of the Animal Care and Use Committee, School of Veterinary Medicine, University of Wisconsin-Madison (V005453). All dogs had written informed consent obtained from their owner.

All dogs included in the ILP Case group were purebred Labrador Retrievers, greater or equal to 8.5 years of age, diagnosed with ILP by a board-certified veterinary surgeon (SJS), board-certified veterinary neurologist (HR, SC), or senior veterinary surgery resident (JHP). Diagnosis was made with a combination of physical exam, clinical signs, neurologic exam, and historical information. All dogs underwent a full neurologic examination at the time of recruitment, supervised by either a board-certified veterinary surgeon (SJS) or a board-certified veterinary neurologist (HR). For inclusion, dogs had to have a history of progressive upper respiratory stridor, which was both exacerbated by exercise and present on examination, as well as pelvic limb weakness.

Dogs in the Control group were from a variety of breeds. Medium- to large-breed dogs that were age matched to the ILP Case population were included. Because ILP is common in Labrador Retrievers, and to avoid preclinical dogs being included as controls, Labrador Retrievers were only included as controls if, at 13.5 years of age, they had no evidence of ILP on examination. To be included as a control, dogs had to 1) be systemically healthy; 2) have no evidence of neuropathy on examination; 3) have no history of respiratory stridor, stertor, cough, or hacking; 4) have no history of regurgitation; and 5) have no history of a bark change. With regards to neurologic assessment, any dog with changes in mentation, altered cranial nerve reflexes/reactions, ataxia, paraparesis or tetraparesis, loss of spinal reflexes in any limb, absent perineal reflex, changes in the cutaneous trunci reflex, or pain on spinal palpation were excluded from the Control group. Dogs were also excluded from the Control group if they developed clinical signs of neuropathy within 6 months of data collection.

For both ILP Case and Control groups, dogs were excluded if they had a history of steroid administration, uncontrolled endocrine disease, chemotherapy administration, or any other conditions known to be associated with neuropathy.

Demographic data, including age, sex, neuter status, breed, and weight, were collected at the time of recruitment. For 97 dogs (n = 40 ILP Cases; 57 Controls), withers height was also collected; for the remaining dogs, mean substitution was used if the dog was a purebred Labrador Retriever, and breed average height was used for all other breeds enrolled.[17] Using body weight and either estimated or true withers height, BMI was calculated as (weight [kg])/(withers height [m])^2.[18] All dogs in both cohorts had 8 mL of blood drawn from a peripheral vein. Blood was collected into EDTA tubes and centrifuged at 1,372 X *g* for 15 minutes at 20 °C. Plasma was then aliquoted and stored at −80 °C. All samples were processed within 1 hour of collection. After blood sample collection, dogs over 12 years of age underwent annual screening for the development of neuropathy.

Nine ILP Cases had 2 plasma samples collected and evaluated at different time points. A new neurologic examination was completed at each plasma collection date. For animals with more than 1 plasma sample obtained at different time points, the most recent

plasma sample was used when comparing between groups and for association with other variables.

The NF-Light Advantage Kit by Single Molecule Array (Simoa) was used to measure pNfL on an HD-1 analyzer, according to the manufacturer's instructions (Quanterix), as previously described.[19] In short, plasma samples were thawed at 21°C, vortexed, and then centrifuged for 5 minutes at 10,000 X *g* with sample diluent. Plasma diluted 1:4 with sample diluent was then bound to paramagnetic beads primed with a human NfL capture antibody. Previous studies[15,16] have demonstrated that human NfL antibodies can be successfully used to detect canine NfL. NfL detection antibodies conjugated with a fluorescent tag were added to NfL-bound beads and incubated. The resultant hydrolysis reaction produces a fluorescent signal proportional to the NfL concentration present in the sample. All measurements were duplicated. Intra- and interassay coefficients of variation were less than 15%.

All statistical analysis was performed with standard software (GraphPad Prism, version 10.1.1 for MacOS[20] or R[19]). Normality was tested using the D'Agostino and Pearson test for all variables, including pNfL, age, weight, height, and BMI. Data are reported as mean ± SD or median (range), as appropriate. Differences between ILP Case and Control groups were first evaluated. A Mann-Whitney *U* test was used to evaluate the differences in pNfL, age, weight, height, and BMI between ILP Case and Control groups. Because age is known to impact pNfL concentrations over a dog's lifespan,[16] an ANOVA was undertaken to investigate the relationship between pNfL concentration, age, BMI, and disease status. The Control group was also evaluated for differences in pNfL concentrations between Control Labrador Retrievers and all other Control medium- to large-breed dogs using a Mann-Whitney *U* test.

Analysis of pNfL concentration with covariates (age, weight, height, and BMI) was undertaken within each group. The relationship between pNfL concentration and age in both ILP Case and Control groups was investigated using linear regression analysis. Spearman rank (S_R) correlation test was used to evaluate correlations between pNfL concentrations and age, weight, height, and BMI within ILP Case and Control groups; correlations were considered strong at $|S_R| \geq 0.70$, moderate at $0.30 \leq |S_R| < 0.7$ and weak at $|S_R| < 0.3$. A bivariate regression model was undertaken to infer the relationship between pNfL concentration with height and weight in the control group. For all noncorrelation analyses, values were considered significant if $P < .05$.

Results

One hundred and twenty-three dogs were included in the study, 62 in the ILP Case Group and 61 in the Control group. Of the 123 dogs, 2 were intact females (2%), 52 were ovariectomized females (42%), 13 were intact males (11%), and 56 were castrated males (46%). All dogs in the ILP Case group (n = 62) were purebred Labrador Retrievers. In the control group, breeds represented included Labrador Retrievers (n = 39), mixed breed (7), Brittany (3), and Pitbull (2) and 1 each of Giant Schnauzer, Siberian Huskey, Bloodhound, English Setter, German Shepherd Dog, Golden Retriever, Boxer, Australian Shepherd, Standard Poodle, and English Springer Spaniel.

pNfL, age, weight, height, and BMI for ILP Case and Control groups were compared. There was no significant difference between pNfL concentrations **(Figure 1)** or age between groups ($P = .36$), although weight ($P < .001$), height ($P = .01$), and BMI ($P < .01$) were higher in the ILP Case group when compared to the Control group **(Table 1)**. There was no difference between pNfL concentrations when the Control group Labrador Retrievers and all other medium- to large-breed dogs in the Control group were compared ($P = .24$; **Supplementary Figure S1**).

To further model differences in pNfL concentration between ILP Case and Control groups, an ANOVA was undertaken to evaluate the relationship between pNfL concentration, age, BMI, and disease status **(Figure 2)**. The estimated parameters for age ($F = 0.129$, $P \leq .720$), for BMI ($F = 0.027$, $P \leq .870$), and disease status ($F = 2.717$, $P \leq .102$) did not show significance at an α-level of 0.05.

Correlations between pNfL concentration and covariates for the ILP Case group and the Control

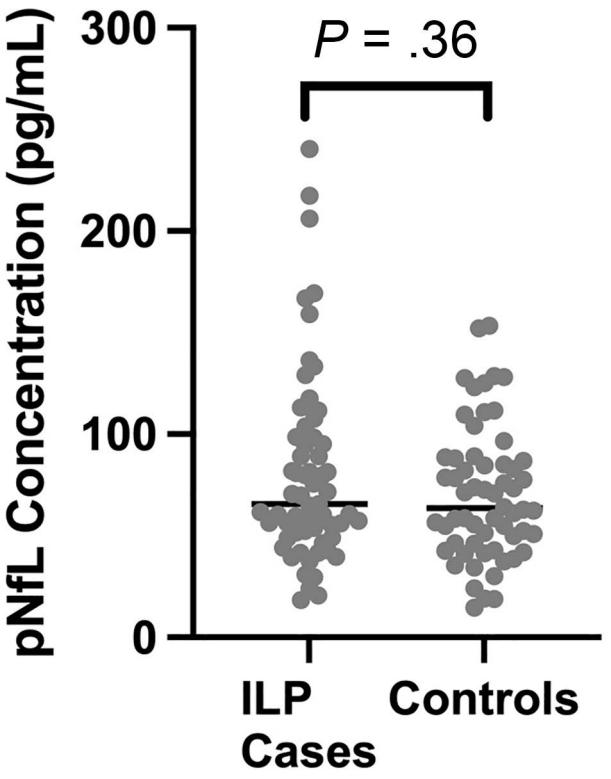

Figure 1—Plasma NfL concentrations were compared between ILP Cases and Controls and illustrated using a scatter plot; lines represent group medians. There was no significant difference in pNfL concentration between ILP Cases and Controls; n = 123 dogs (62 ILP Cases; 61 Controls). ILP = Idiopathic laryngeal paralysis. pNfL = Plasma neurofilament light chain.

Table 1—pNfL concentration, age, weight, estimated height, and estimated BMI in ILP Cases and Controls with significance values.

	ILP Cases	Control	*P* value
pNfL (pg/mL)	65.6 (18.4–240.0)	63.5 (14.7–153.3)	.36
Age (y)	12.28 ± 1.36	11.79 ± 1.72	.09
Weight (kg)	34.77 ± 6.48	29.97 ± 7.16	*.0002*
Height (m)	0.58 (0.52–0.72)	0.57 (0.44–0.68)	*.01*
BMI (kg/m^2)	95.65 (60.19–142.21)	92.26 (60.97–149.69)	*.004*

n = 123 dogs (62 ILP Cases; 61 Controls).
BMI = Body mass index. ILP = Idiopathic laryngeal paralysis. pNfL = Plasma neurofilament light chain.
Significant results are shown in italics.

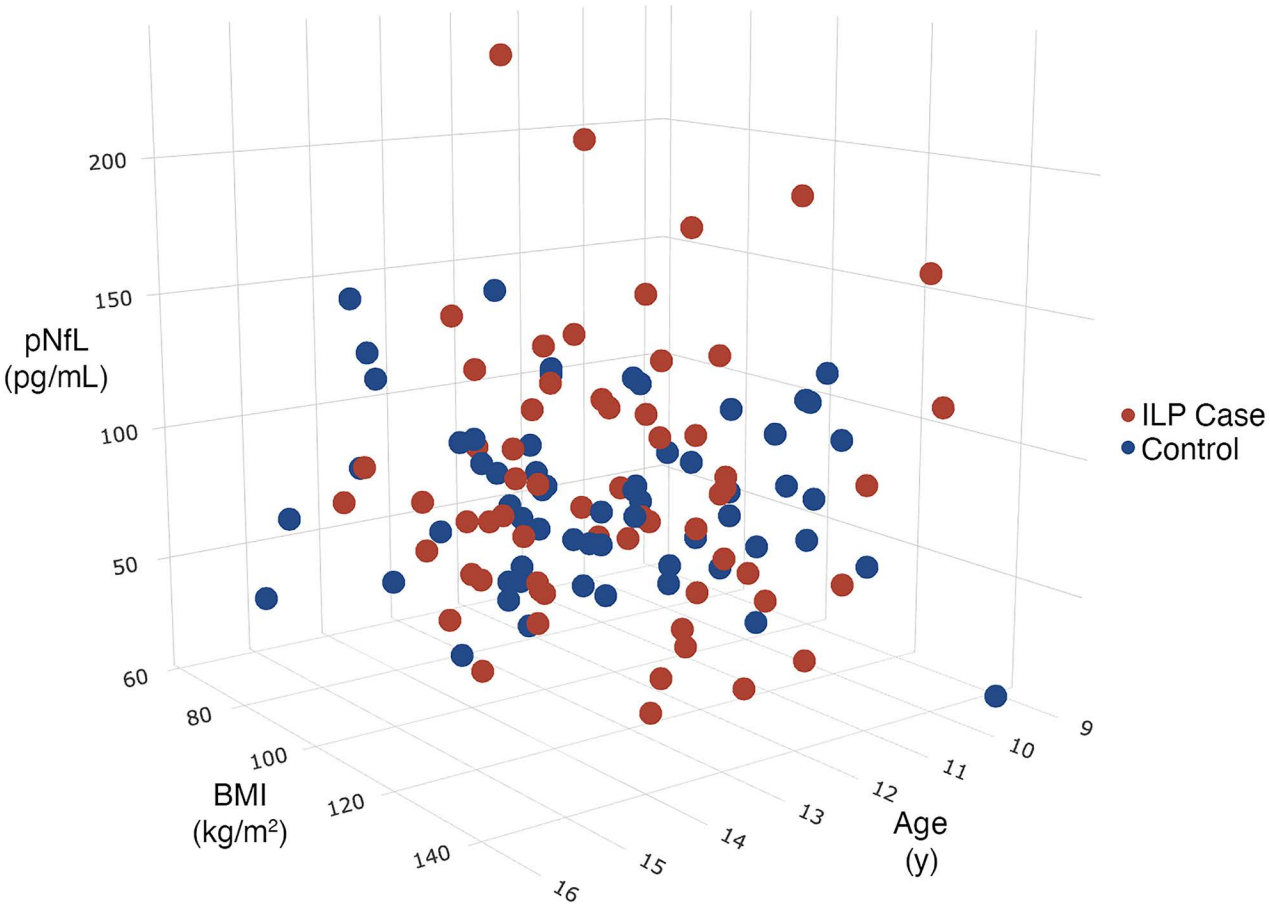

Figure 2—A scatter plot showing the relationship between pNfL concentration, age, estimated BMI, and disease status in all dogs. Within the plot, red dots represent ILP Cases and blue dots represent Controls. There were no significant relationships between pNfL concentration, age, and estimated BMI in either ILP Cases or Controls; n = 123 dogs (62 ILP Cases; 61 Controls). BMI was calculated as body weight/[withers height^2].[21] BMI = Body mass index. ILP = Idiopathic laryngeal paralysis. pNfL = Plasma neurofilament light chain.

group were evaluated. For the ILP Case group, there were weak nonsignificant correlations between pNfL and age (S_R = −0.05, *P* = .68), weight (S_R = −0.13, *P* = .32), height (S_R = 0.05, *P* = .68), and BMI (S_R = −0.12, *P* = .36). For the Control group, there were weak nonsignificant correlations between pNfL and age (S_R = 0.19, *P* = 0.14) and BMI (affected: S_R = −0.046, *P* = .23; control: S_R = −0.14, *P* = .73), although moderate significant negative correlations were noted between pNfL and weight (S_R = −0.30, *P* = .02) and height (S_R = −0.38, *P* = .003). When a bivariate regression model was undertaken to infer the relationship between pNfL concentration with height and weight in the control group, height (*P* = .03), showed a significant relationship, but weight (*P* = .79) was not found to influence pNfL values **(Supplementary Table S1)**. When a linear regression of age versus pNfL concentrations was undertaken for ILP Cases and Controls, a positive linear relationship was seen in the Control group and a negative linear relationship was seen in the ILP Case group **(Figure 3)**.

Nine ILP Case dogs had serial plasma sample collections. The time between sample collections ranged from 63 to 601 days. pNfL concentrations did not

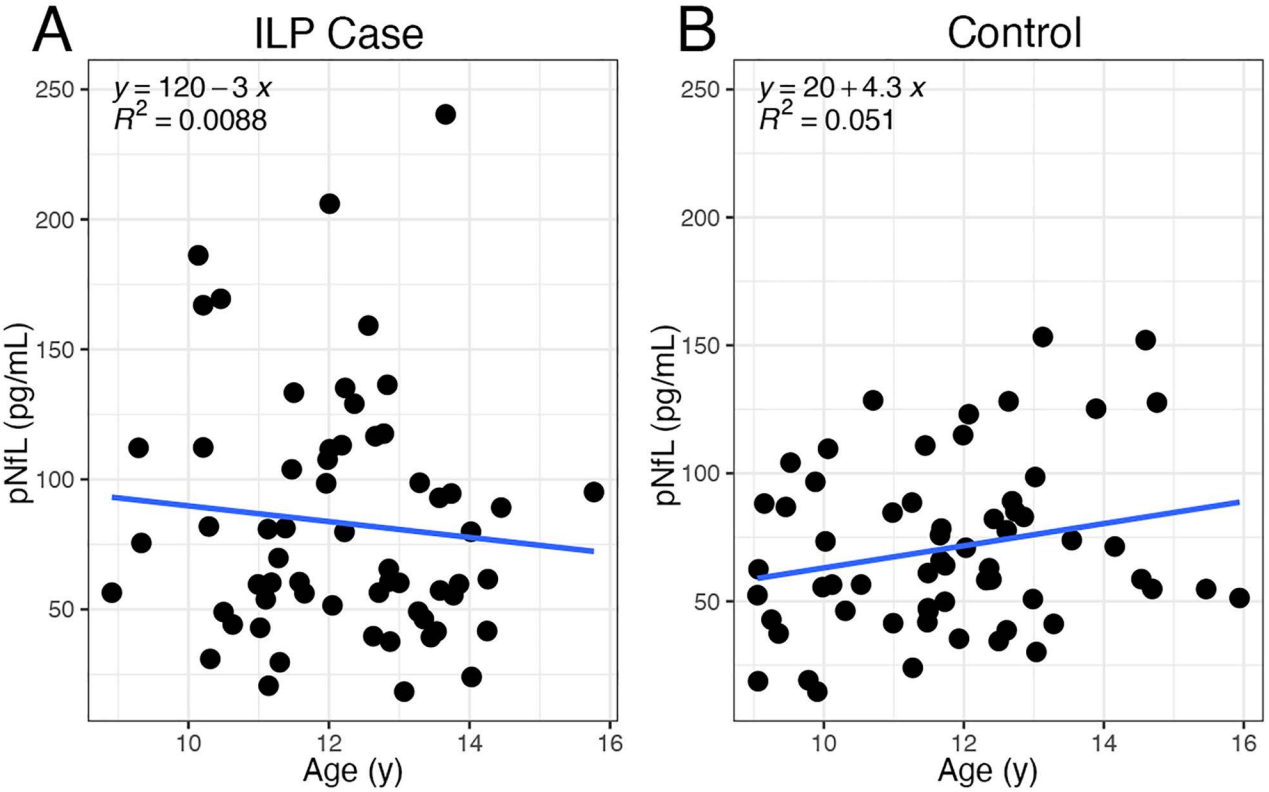

Figure 3—Regression plots illustrate the relationship between pNfL concentration and age in ILP Cases, Controls, and all dogs enrolled in the study. A—pNfL concentration was not significantly associated with age in ILP Cases, although a negative trend was noted. B—pNfL concentration was also not significantly associated with age in Controls although a positive trend was noted; n = 123 dogs (62 ILP Cases; 61 Controls). ILP = Idiopathic laryngeal paralysis. pNfL = Plasma neurofilament light chain.

consistently increase or decrease regardless of the time between measurements **(Supplementary Table S2)**.

Discussion

The aim of this study was to investigate pNfL concentrations in ILP-affected Labrador Retrievers. We found that pNfL concentrations were not different between ILP-affected Labrador Retrievers and an age-matched control population of medium- to large-breed dogs, and thus pNfL concentration is not a biomarker reflective of disease status in Labrador Retrievers affected with ILP. We therefore reject our first hypothesis that pNfL concentrations would be elevated in ILP-affected Labrador Retrievers. We partially reject our second hypothesis, as height and weight did correlate with pNfL concentration in the Control population but not the ILP Case population.

Previous studies[15,16] have established that pNfL concentration can be measured effectively in healthy dogs and that pNfL concentration is affected by age, height, and weight in healthy Labrador Retrievers, and for this reason, these covariates were investigated in the present study in detail. Dogs with canine cognitive dysfunction and degenerative myelopathy, conditions that affect the CNS, have increased levels of pNfL compared to healthy controls.[15] In humans, pNfL has also been shown to increase in conditions affecting the CNS, such as Parkinson disease, multiple sclerosis, and amyotrophic lateral sclerosis, among others.[12,13,21,22]

Studies[23] investigating pNfL values in peripheral neuropathies are more limited, and associations between pNfL values and disease status are variable between disease types. For example, over 90 subtypes of CMT exist. pNfL concentrations have only been investigated in a limited number of these CMT subtypes with varying results. pNfL concentration is increased in some CMT subtypes (CMT1A, CMT1B, CMT2A, CMT4B2, CMT4C, and CMT1X), and these increases are related to disease severity; in other CMT subtypes (CMT2E) pNfL concentration is not inc reased.[14,24,25] Overall, increases in pNfL values seen in patients with CMT are often small enough to call into question the clinical utility of the biomarker.[25] It is also unclear what pathologic features of varying peripheral neuropathies lend themselves to pNfL increases. The results of this study suggest that ILP, like CMT2E, is a peripheral neuropathy that does not result in consistent or meaningful alterations in pNfL.

In this study, the ILP Case population was limited to Labrador Retrievers. It is unclear whether ILP is a single disease condition or, like many inherited peripheral neuropathies in humans, a common clinical presentation resulting from a variety of etiologies. For this reason, limiting the ILP Case population to a

single breed was undertaken to minimize phenotypic and pathologic variation within the ILP Case group.[16]

For the Control group, Labrador Retrievers along with medium- to large-sized dogs were used. As the prevalence of ILP is substantial within the Labrador Retriever breed and the age of clinical onset can be late in life, only utilizing Labrador Retrievers for the control population would not have 1) resulted in a robust sample size, 2) enabled a control population that does not have preclinical ILP-affected dogs, and thus 3) enable an age-matched control design. For these reasons, additional medium- to large-breed dogs were recruited. Previous work[15] established that significant differences in pNfL concentration between dogs with degenerative myelopathy and a control population could be found using a variety of dog breeds without issue. Similarly, we found no significant differences in pNfL concentrations between Labrador Retrievers and other medium- to large mixed-breed dogs in the Control group, supporting the use of additional age-matched dogs that were reasonably size matched (Table 1). In humans, the evaluation of pNfL across ethnic populations has not identified ancestry to be influential.[26,27] Selection of control dogs approximately the same size as Labrador Retrievers was undertaken as prior work[16] has shown height to be associated with pNfL concentrations. However, additional studies investigating pNfL concentrations across different breeds would be needed to further investigate whether various breed phenotypes relate to pNfL concentrations.

The Labrador Retrievers included in the Control group were enrolled in a prior study[2,6] for which plasma had been collected earlier in life; for these dogs, if the individual Labrador Retriever did not have evidence of neuropathy at 13.5 years of age, the previously collected samples were included in the Control group. An age cutoff of 13.5 years was considered a conservative value to eliminate preclinical ILP-affected Labradors from being assigned to the Control group, which is a substantial risk when working on late-onset disease.[2,6]

It is established that pNfL concentration increases with age in humans and dogs.[15,16,24] However, in our study, we failed to find a significant association between age and pNfL concentration. In this study, a positive but nonsignificant relationship between age and pNfL concentration was present in the Control group, indicating that to some degree pNfL concentration did increase in this population. The lack of association between age and pNfL concentration is likely due to the relatively small age range included as part of this study's design (9 to 16 years old), particularly given the large concentration of dogs between 10 and 12 years of age. It is interesting that linear regression showed that the pNfL concentration had a negative slope for the ILP Cases; this finding was unexpected and requires further investigation.

In addition to investigating whether pNfL concentration could differentiate ILP Cases from Controls, we collected repeat samples in ILP Case dogs to determine whether pNfL concentrations altered within individuals over time. We did not detect any significant difference in serial samples in any individual despite a wide range of sampling windows. This finding agrees with a longitudinal study[25] investigating pNfL trends over 6 years in patients with CMT, wherein most CMT subtypes evaluated showed no changes in pNfL concentrations over time. Further studies conducted using a larger sample size, more sampling points and performed over a longer period would be necessary to determine the utility of pNfL concentration trends in dogs with ILP.

As in previous studies,[16] Control group pNfL concentration was negatively correlated with height and weight, while BMI did not correlate with pNfL concentrations. Because height and weight are related phenotypic attributes, a regression was undertaken and indicated that in the present study's Control group, height, not weight, was influential on pNfL. The relationship between height, weight, and pNfL across age groups has not been thoroughly investigated in dogs or people. A correlation between increased pNfL and decreased weight was seen in a prior study[16] of healthy Labrador Retrievers, where this finding was consistent across age groups, although the relationship between pNfL concentration and weight was not seen in another study[15] that used multiple breeds. Overall, further work to establish normal variations of pNfL concentration in healthy dogs across multiple breeds and a range of heights and weights would be of benefit given the broad range of height phenotypes within the canine species.

BMI and blood volume appear to influence pNfL concentration in human populations, although correlation studies[28–30] have shown conflicting results depending on the patient population being investigated. Neither the current study nor a previous study[16] investigating a relationship between pNfL and BMI in dogs detected any relationship between pNfL concentration and BMI. Further investigations into more heterogeneous populations of dogs would be needed to understand whether BMI or blood volume impacts pNfL concentrations in dogs.

There were limitations to this study. A laryngeal examination was not a requirement for phenotyping in this study; none of the Control dogs had a laryngeal examination, and 60% of ILP Case dogs were not examined in this way. Laryngeal examination under light anesthesia is often used to diagnose ILP.[31–33] However, false positive and false negative diagnoses can occur. We considered laryngeal examinations within an overall clinical picture for each individual dog. Not all ILP-affected dogs had a glottic opening surgery. The risk of undertaking a laryngeal examination in ILP cases not otherwise undergoing general anesthesia was considered an unnecessary risk given these dogs' overall clinical picture. The use of laryngeal examinations to confirm laryngeal function in the Control population of aged dogs was also not undertaken given the stringency by which the Control group was selected. However, the lack of a direct airway examination to support the clinical

phenotyping of dogs in this study may have led to inaccurate case/control classification in some dogs.

In conclusion, pNfL concentration does not appear to be a robust, sensitive measure for detection or disease progression of ILP in Labrador Retrievers. However, pNfL can be reliably measured in dogs with a simple blood draw and has the potential as a useful biomarker for other conditions. Further studies investigating the utility of pNfL as a biomarker in other companion animal neurogenerative diseases are warranted.

Acknowledgments

The authors acknowledge Alexander Rossor of the Department of Neurodegenerative Disease, UCL Institute of Neurology, for his input and support in this work.

Disclosures

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

No AI-assisted technologies were used in the generation of this manuscript.

Funding

This work was supported by the National Institutes of Health (K01OD019743-01A1, T32OD010423) and the UK Dementia Research Institute at University College London (UCL). HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (Nos. 2023-00356, 2022-01018, and 2019-02397); the European Union's Horizon Europe Research and Innovation Programme under grant No. 101053962; Swedish State Support for Clinical Research (No. ALFGBG-71320); the Alzheimer Drug Discovery Foundation (ADDF) USA (No. 201809-2016862); the AD Strategic Fund and the Alzheimer's Association (Nos. ADSF-21-831376-C, ADSF-21-831381-C, and ADSF-21-831377-C); the Bluefield Project; Cure Alzheimer's Fund, the Olav Thon Foundation, the Erling-Persson Family Foundation; Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (No. FO2022-0270); the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant No. 860197 (MIRIADE); the European Union Joint Programme-Neurodegenerative Disease Research (JPND2021-00694); the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre; and the UK Dementia Research Institute at UCL (UKDRI-1003).

References

1. Sample SJ, Stilin A, Binversie EE, Baker LA, Hardie RJ. Late-onset laryngeal paralysis: 0wner perception of quality of life and cause of death. *Vet Medicine Sci.* 2020;6(3):306–313. doi:10.1002/vms3.240
2. Thieman KM, Krahwinkel DJ, Sims MH, Shelton GD. Histopathological confirmation of polyneuropathy in 11 dogs with laryngeal paralysis. *J Am Anim Hosp Assoc.* 2010;46(3):161 167. doi:10.5326/0460161
3. Granger N. Canine inherited motor and sensory neuropathies: an updated classification in 22 breeds and comparison to Charcot-Marie-Tooth disease. *Vet J.* 2011;188(3):274 285. doi:10.1016/j.tvjl.2010.06.003
4. Stanley BJ, Hauptman JG, Fritz MC, Rosenstein DS, Kinns J. Esophageal dysfunction in dogs with idiopathic laryngeal paralysis: a controlled cohort study. *Vet Surg.* 2010;39(2):139 149. doi:10.1111/j.1532-950x.2009.00626.x
5. Letko A, Minor KM, Friedenberg SG, et al. A CNTNAP1 missense variant is associated with canine laryngeal paralysis and polyneuropathy. *Genes (Basel)* 2020;11(12):1426. doi:10.3390/genes11121426
6. MacPhail CM. Laryngeal disease in dogs and cats an update. *Vet Clin North Am Small Animal Pract.* 2020;50(2):295–310. doi:10.1016/j.cvsm.2019.11.001
7. Bookbinder LC, Flanders J, Barry JS, Cheetham J. Idiopathic canine laryngeal paralysis as one sign of a diffuse polyneuropathy: an observational study of 90 cases (2007–2013). *Vet Surg.* 2016;45(2):254 260. doi:10.1111/vsu.12444
8. Palermo G, Mazzucchi S, Vecchia AD, et al. Different clinical contexts of use of blood neurofilament light chain protein in the spectrum of neurodegenerative diseases. *Mol Neurobiol.* 2020;57(11):4667–4691. doi:10.1007/s12035-020-02035-9
9. Yuan A, Rao MV, Veeranna, Nixon RA. Neurofilaments and neurofilament proteins in health and disease. *Csh Perspect Biol.* 2017;9(4):a018309. doi:10.1101/cshperspect.a018309
10. Altmann P, Ponleitner M, Rommer PS, et al. Seven day pre-analytical stability of serum and plasma neurofilament light chain. *Sci Rep.* 2021;11(1):11034. doi:10.1038/s41598-021-90639-z
11. Wang SY, Chen W, Xu W, et al. Neurofilament light chain in cerebrospinal fluid and blood as a biomarker for neurodegenerative diseases: a systematic review and meta-analysis. *J Alzheimer's Dis.* 2019;72(4):1353–1361. doi:10.3233/jad-190615
12. Verde F, Steinacker P, Weishaupt JH, et al. Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis. *J Neurology Neurosurg Psychiatry.* 2018;90(2):157–164. doi:10.1136/jnnp-2018-318704
13. Kuhle J, Kropshofer H, Haering DA, et al. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. *Neurology.* 2019;92(10):e1007–e1015. doi:10.1212/wnl.0000000000007032
14. Millere E, Rots D, Simrén J, et al. Plasma neurofilament light chain as a potential biomarker in Charcot-Marie-Tooth disease. *Eur J Neurol.* 2021;28(3):974–981. doi:10.1111/ene.14689
15. Panek WK, Gruen ME, Murdoch DM, et al. Plasma neurofilament light chain as a translational biomarker of aging and neurodegeneration in dogs. *Mol Neurobiol.* 2020;57(7):3143–3149. doi:10.1007/s12035-020-01951-0
16. Perino J, Patterson M, Momen M, et al. Neurofilament light plasma concentration positively associates with age and negatively associates with weight and height in the dog. *Neurosci Lett.* 2021;744:135593. doi:10.1016/j.neulet.2020.135593
17. American Kennel Club. Dog breeds. Accessed December 14, 2022. https://www.akc.org/dog-breeds/
18. Comhaire FH, Snaps F. Comparison of two canine registry databases on the prevalence of hip dysplasia by breed and the relationship of dysplasia with body weight and height. *Am J Vet Res.* 2008;69(3):330–333. doi:10.2460/ajvr.69.3.330
19. R Core Team. *The R Project for Statistical Computing*. The R Foundation; 2021. Accessed July 28, 2022. https://www.R-project.org/

20. *GraphPad Prism*. GraphPad Software. Accessed January 22, 2023. www.graphpad.com
21. Gaetani L, Blennow K, Calabresi P, Filippo MD, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. *J Neurology Neurosurg Psychiatry.* 2019;90(8):870–881. doi:10.1136/jnnp-2018-320106
22. Olsson B, Portelius E, Cullen NC, et al. Association of cerebrospinal fluid neurofilament light protein levels with cognition in patients with dementia, motor neuron disease, and movement disorders. *JAMA Neurol.* 2019;76(3):318. doi:10.1001/jamaneurol.2018.3746
23. Pisciotta C, Shy ME. Chapter 42 – Neuropathy. *Handb Clin Neurol.* 2018;148:653–665. doi:10.1016/b978-0-444-64076-5.00042-9
24. Sandelius Å, Zetterberg H, Blennow K, et al. Plasma neurofilament light chain concentration in the inherited peripheral neuropathies. *Neurology.* 2018;90(6):e518 e524. doi:10.1212/wnl.0000000000004932
25. Rossor AM, Kapoor M, Wellington H, et al. A longitudinal and cross-sectional study of plasma neurofilament light chain concentration in Charcot-Marie-Tooth disease. *J Peripher Nerv Syst.* 2022;27(1):50–57. doi:10.1111/jns.12477
26. Brickman AM, Manly JJ, Honig LS, et al. Plasma p-tau181, p-tau217, and other blood-based Alzheimer's disease biomarkers in a multi-ethnic, community study. *Alzheimer's Dement.* 2021;17(8):1353–1364. doi:10.1002/alz.12301
27. O'Bryant SE, Petersen M, Hall J, Johnson LA, HABS-HD Study Team. Medical comorbidities and ethnicity impact plasma Alzheimer's disease biomarkers: important considerations for clinical trials and practice. *Alzheimer's Dement.* 2023;19(1):36–43. doi:10.1002/alz.12647
28. Manouchehrinia A, Piehl F, Hillert J, et al. Confounding effect of blood volume and body mass index on blood neurofilament light chain levels. *Ann Clin Transl Neurol.* 2020;7(1):139–143. doi:10.1002/acn3.50972
29. Benkert P, Meier S, Schaedelin S, et al. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. *Lancet Neurol.* 2022;21(3):246–257. doi:10.1016/s1474-4422(22)00009-6
30. Windon C, Iaccarino L, Mundada N, et al. Comparison of plasma and CSF biomarkers across ethnoracial groups in the ADNI. *Alzheimer's Dement Diagn Assess Dis Monit.* 2022;14(1):e12315. doi:10.1002/dad2.12315
31. Kapaldo N, McMurphy R, Hodgson D, Roush J, Berke K, Klocke E. Laryngeal function in normal dogs administered isoflurane following partial clearance of alfaxalone or propofol. *Vet Anaesth Analg.* 2021;48(4):493–500. doi:10.1016/j.vaa.2021.03.009
32. Labuscagne S, Zeiler GE, Dzikiti BT. Effects of chemical and mechanical stimulation on laryngeal motion during alfaxalone, thiopentone or propofol anaesthesia in healthy dogs. *Vet Anaesth Analg.* 2019;46(4):435–442. doi:10.1016/j.vaa.2018.12.010
33. Radkey DI, Hardie RJ, Smith LJ. Comparison of the effects of alfaxalone and propofol with acepromazine, butorphanol and/or doxapram on laryngeal motion and quality of examination in dogs. *Vet Anaesth Analg.* 2018;45(3):241–249. doi:10.1016/j.vaa.2017.08.014

Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org