Neurofilament light plasma concentration positively associates with age and negatively associates with weight and height in the dog

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ABSTRACT

Plasma neurofilament light chain (pNfL) concentration is a biomarker for neuroaxonal injury and degeneration and can be used to monitor response to treatment. Spontaneous canine neurodegenerative diseases are a valuable comparative resource for understanding similar human conditions and as large animal treatment models. The features of pNfL concentration in healthy dogs is not well established. We present data reporting basic pNfL concentration trends in the Labrador Retriever breed. Fifty-five Labrador Retrievers were enrolled. pNfL concentration was measured and correlated to age, sex, neuter status, height, weight, body mass index, and coat color. We found increased pNfL with age (P < 0.0001), shorter stature (P = 0.009) and decreased body weight (P < 0.001). These are similar to findings reported in humans. pNfL concentration did not correlate with sex, BMI or coat color. This data further supports findings that pNfL increase with age in a canine population but highlights a need to consider weight and height when determining normal pNfL concentration in canine populations.

1. Introduction

Neurofilaments (NF) provide structural support for axons and contribute to regulation of myelinated axonal diameter. Neurofilaments are comprised of 4 subunits, including neurofilament light chain (NfL), neurofilament middle chain, neurofilament heavy chain, and α-inter-nexin [1]. Of these, NfL is the most abundant and soluble, and is stable in biofluids [2]. Low levels of NfL are constantly released from axons, and levels of NfL release increase with age [3, 4]. Higher levels of NfL are also released in neurodegenerative and neuroinflammatory diseases, as well as in cases of trauma or vascular disease [1].

NfL is of interest as a biomarker for neurodegenerative disease due to ease of acquisition in blood samples. Use of NfL concentration in human neurodegenerative disease has focused on improvement of diagnostic accuracy [5, 6], as a prognostic biomarker [3, 5], and as a means for monitoring disease progression and potentially response to treatment. NfL measurements in blood have substantial promise for evaluating response to novel disease-modifying therapies in clinical trials [7].

Canine models of neurodegenerative diseases are valuable for investigation of pathologic disease progression and for studies of disease-modifying therapy. For example, degenerative myelopathy (DM) is a relatively common motor neuron disease in dogs that is genetically and clinically similar to ALS, and is currently used for drug treatment trials [8, 9]. Canine models of Duchenne muscular dystrophy [10], globoïd cell leukodystrophy [11], and Alzheimer’s disease [12] are also under investigation as neurodegenerative disease models.

Use of plasma NfL (pNfL) concentration in dog models of neurodegenerative disease has potential for evaluation of response to therapy. Only one study is available reporting on the relationship between pNfL concentration and age in normal dogs, which included 34 healthy dogs of various breeds, and found pNfL concentration increased with age [7]. The objective of the present study was to determine the relationship between pNfL concentration and age, sex, coat color, height, weight and body mass index in a large homogeneous population of healthy purebred dogs.
2. Materials and methods

Owners of purebred Labrador Retrievers were recruited from the UW Veterinary Care Hospital at the University of Wisconsin-Madison between 2016 and 2019. All procedures were conducted with the approval of the Animal Care & Use Committee, School of Veterinary Medicine, University of Wisconsin-Madison (V005453). Dogs were recruited with informed written consent from each owner.

For inclusion, dogs had to be systemically healthy pure-bred Labrador Retrievers with no evidence of neuropathy on examination. Dogs were excluded if they had a history of chemotherapy, steroid administration, uncontrolled endocrine disease, any other condition associated with neuropathy, or were found to develop clinical evidence of neuropathy within 6 months of sample collection. Features of neuropathy that were deemed worthy of exclusion included: change in mentation, alterations in cranial nerve reflexes or reactions, development of ataxia, human 

3. Results

Plasma was collected from 59 Labrador Retrievers. Four dogs were excluded due to development of clinical neuropathy within 6 months of initial plasma sample collection. Of the 55 Labrador Retrievers included in the final analysis, 3 were intact males (5%), 17 were castrated males (31%), 5 were intact females (9%), and 30 were ovarioectomized females (55%). All three primary coat colors were represented, including yellow: n = 23 (42%), chocolate: n = 11 (20%), and black: n = 21 (38%). Median age was 9.1 years (range 1.4–14.8 years). Median pNfL level was 42.8 pg/mL (range 5.2–129.0 pg/mL). Median weight was 32.8 kg (range 22.0–56.0 kg) (Fig. 1A). Mean withers height was 0.57 ± 0.005 m (Fig. 1B). Median BMI was 98.8 kg/m² (range 64.5–154.8 kg/m²) (Fig. 1C).

Results of Spearman Rank correlations between pNfL concentration and recorded variables are displayed (Table 1). pNfL concentration had a significantly and moderately positive correlation with age ($R_p = 0.68$, $P < 0.0001$) (Fig. 2). Height and weight had weak and moderate negative correlations, respectively, with pNfL concentration ($S_R = -0.35$, $P < 0.001$; $S_R = -0.51$, $P < 0.0001$, respectively) (Fig. 2), with height and weight being correlated with each other ($S_R = 0.58$, $P < 0.001$). BMI did not correlate significantly with pNfL concentration ($S_R = -0.23$, $P = 0.09$) (Table 1, Fig. 2). There was no significant correlation between pNfL concentration and sex ($S_R = 0.13$, $P = 0.36$), coat color ($S_R = 0.19$, $P = 0.17$), neuter status ($S_R = -0.08$, $P = 0.55$), neuter status in males ($S_R = -0.23$, $P = 0.33$), or neuter status in females ($S_R = 0.02$, $P = 0.93$) (Table 1, Table S1).

When the population was segregated by sex, the association between age and pNfL concentration remained significant and positive (Table S1). Sex differences were seen between correlations with weight, height, and BMI. In females, weight and height both negatively and moderately correlated with pNfL concentration ($S_R = -0.55$, $P = 0.006$; $S_R = -0.55$, $P = 0.006$), although BMI did not ($S_R = -0.03$, $P = 0.87$). In males, neither height nor weight correlated with pNfL concentration, while BMI had a moderate negative correlation ($S_R = -0.52$, $P = 0.02$).

When pNfL concentration was evaluated against variables obtained in a subset of dogs under 11 years of age (n = 39), the same significant associations were noted. pNfL concentration strongly correlated with age ($S_R = 0.74$, $P < 0.0001$) and moderately correlated with weight and height ($S_R = -0.45$, $P = 0.005$; $S_R = -0.45$, $P = 0.004$, respectively). Other variables did not correlate with pNfL concentration, including sex ($S_R = 0.22$, $P = 0.17$), coat color ($S_R = 0.36$, $P = 0.15$) and BMI ($S_R = 0.24$, $P = 0.14$) (Table 1). Results from analysis of dogs under 5 years of age and dogs between 5–11 years of age are presented in Table S2.

Correlations between variables obtained and age were determined (Table 1, Fig. 1). Weight was negatively and moderately correlated with age ($S_R = -0.44$, $P < 0.001$), as was BMI ($S_R = -0.39$, $P = 0.004$). No other significant correlations with age were noted.

Due to the individual correlations between age and weight, as well as both age and weight correlating to pNfL concentration, a bivariate linear regression model was used to further investigate the interactions between these variables. pNfL concentration was influenced by both increasing age ($P < 0.0001$) and decreasing weight ($P = 0.04$) (Table S3). This analysis was repeated to further investigate the relationship between age, BMI and pNfL concentration, confirming that increasing age ($P < 0.001$), but not decreasing BMI ($P = 0.09$), was associated with pNfL concentration (Table S3).

4. Discussion

The purpose of this study was to define the relationship between pNfL concentration with age, sex, body mass index, height, weight, and coat color in a normal homogeneous population of purebred Labrador Retriever dogs. It is established that plasma and CSF NfL concentration...
are biomarkers for neurodegenerative diseases in humans [5,14]. This work is important because pNfL levels are an informative biomarker that can be used to study dog models of neurodegenerative diseases. Specific considerations associated with dog phenotypic attributes must be defined. The correlation of pNfL and age has been shown in human samples, indicating that age corrections need to be optimally employed to assess pNfL concentration as a biomarker in neurodegenerative disease [14,15]. Our results support the hypothesis that pNfL concentration increases with age and is not influenced by sex or coat color. Results did not support our second hypothesis, as no significant association was seen between pNfL concentration and BMI, although associations were seen between pNfL concentration and BMI, although associations were seen between pNfL concentration and height and weight.

Dogs are an important large animal model for numerous neurodegenerative diseases, providing insight into progression of disease and response to novel treatment therapies [12]. While many dog models are laboratory-bred, it is becoming increasingly common to study spontaneous models of neurodegenerative diseases through use of client-owned animals. This results in a larger degree of variance regarding age, sex and body condition [7]. For spontaneous genetic diseases in dogs, breed is also an important consideration; disease risk is highly breed-dependent, and phenotypic and genomic variance between breeds is substantial [16].

Use of a single purebred population helped minimize variance due to genetics, improving insight into phenotypic variables on pNfL concentration in dogs. We found that in the Labrador Retriever, pNfL concentration significantly increased with age, which is a well-documented feature of pNfL levels in humans. This finding is in agreement with recent work evaluating pNfL concentration in 34 clinically normal dogs of varying breeds [7]. Our method for measuring pNfL differed from this earlier dog study, preventing direct comparison of plasma concentrations. Another previous study investigating the relationship between age and pNfL concentration in dogs found a lack of significant changes in pNfL concentration within the first 2 years of life [17].

We chose to analyze data from all dogs as well the subset of dogs under 11 years of age. The average life expectancy for Labrador Retrievers in the USA is not known, although limited data has reported an average life expectancy of 13.2 years [18]; we, therefore, chose 11 years of age to be a conservative cut off for “aged” status. As with human beings, dogs can develop cognitive dysfunction in their senior years, and dogs with cognitive dysfunction have increased pNfL levels [7]. The dogs recruited for this study were not extensively evaluated for cognitive dysfunction, and thus evaluating a younger subset of dogs independently was deemed prudent. Results from the younger subset of dogs mirrored the results seen when all dogs were analyzed, suggesting that if any aged dogs were experiencing cognitive dysfunction it did not substantially influence results.

We undertook evaluation of dogs broken into more specific age groups, including young adults, adults and aged dogs. Correlations seen in the entire population were present in the adult dog group. However, the same correlations with pNfL did not hold for all variables when only dogs <5 years of age (1.4–3.8 years) or dogs >11 years of age (11.2–14.8 years) were considered. This is likely due to the limited age span of these subgroups and low sample sizes (Table S2).

Purebred Labrador Retrievers have a wide range of heights, making body weight an inaccurate method to estimate body condition and a poor proxy for body size. We determined BMI as a measure of each dog’s body condition. In humans, neuropathy is an identified risk factor associated with obesity [19], and pNfL concentration correlated with BMI and blood volume (BV) when age and sex were considered in an analysis of 662 human controls [20]. We did not find pNfL concentration correlated with BMI (Fig. 2). This may reflect relatively low sample size and presence of differing hormonal sex status (intact male, intact female, castrated male and ovarioectomized female) in the sample population, making detailed analysis of sex effects on pNfL concentrations difficult to interpret. However, when considered alone, male dogs had a moderate negative correlation with BMI while female dogs did not. Future work with increased sample sizes across all sexes would allow for more detailed analysis of the relationship between pNfL levels and BMI with adjustments made for sex and age.

In this Labrador population, dogs with a shorter stature had increased pNfL concentration. The relationship between height and pNfL concentration has not previously been investigated in dogs or people. However, both BMI and BV were found to correlate with pNfL concentration in humans [20]; both BMI and BV calculations include height and weight. This is relevant as the height correlation seen in this study may similarly be the result of decreased blood volume in shorter dogs. Alternatively, this correlation with height may be due to shorter

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### Table 1

Results of correlations between variables measured, pNfL concentration, and age are presented. pNfL = plasma neurofilament light concentration.

<table>
<thead>
<tr>
<th>Variable</th>
<th>pNfL All Dogs (n = 55)</th>
<th>pNfL Dogs &lt;11 years (n = 39)</th>
<th>Age (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pNfL (pg/mL)</td>
<td>$S_R = 0.68$, $P &lt; 0.0001$</td>
<td>$S_R = 0.48$, $P &lt; 0.0001$</td>
<td>$S_R = 0.68$, $P &lt; 0.0001$</td>
</tr>
<tr>
<td>Age (years)</td>
<td>$S_R = 0.13$, $P = 0.36$</td>
<td>$S_R = 0.22$, $P = 0.17$</td>
<td>$S_R = 0.01$, $P = 0.92$</td>
</tr>
<tr>
<td>Sex</td>
<td>$S_R = 0.08$, $P = 0.55$</td>
<td>$S_R = 0.0$, $P = 0.99$</td>
<td>$S_R = 0.01$, $P = 0.92$</td>
</tr>
<tr>
<td>Neuter status</td>
<td>$S_R = 0.19$, $P = 0.17$</td>
<td>$S_R = 0.36$, $P = 0.15$</td>
<td>$S_R = 0.03$, $P = 0.61$</td>
</tr>
<tr>
<td>Coat Color</td>
<td>$S_R = 0.51$, $P = 0.001$</td>
<td>$S_R = 0.45$, $P = 0.005$</td>
<td>$S_R = 0.44$, $P = 0.001$</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>$S_R = 0.35$, $P = 0.009$</td>
<td>$S_R = 0.45$, $P = 0.004$</td>
<td>$S_R = 0.12$, $P = 0.40$</td>
</tr>
<tr>
<td>Height (m)</td>
<td>$S_R = 0.23$, $P = 0.09$</td>
<td>$S_R = 0.24$, $P = 0.14$</td>
<td>$S_R = 0.39$, $P = 0.004$</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>$S_R = 0.68$, $P &lt; 0.0001$</td>
<td>$S_R = 0.48$, $P &lt; 0.0001$</td>
<td>$S_R = 0.68$, $P &lt; 0.0001$</td>
</tr>
</tbody>
</table>

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Fig. 1. Distribution of weight, withers height, and BMI in study population. Box and whiskers plots showing the distribution of A) weight, B) withers height, and C) body mass index (BMI). BMI was thus calculated as BMI = (body weight / (wither height$^2$)). [13]. n = 55 dogs.
Labradors having a more chondrodysplastic phenotype, which predisposes dogs to Hanson Type II intervertebral disc degeneration [21]; magnetic resonance imaging would be needed to evaluate this further. Evaluation of multiple breeds with varying chondrodystrophic status would be prudent given the potentially heterogenous populations of dogs used for clinical studies.

The correlations between increased pNfL concentration and decreased weight are also interesting. We found that both age and weight independently influenced pNfL concentration. Given that shorter dogs typically weigh less, the association between pNfL concentration and weight likely reflects the association between pNfL concentration and height. The only prior study to evaluate weight and pNfL concentration in dogs used multiple breeds and did not identify this relationship [7]. An association between weight and pNfL levels has not been defined in humans, although individuals with metabolic syndrome have a higher prevalence of peripheral neuropathy [19]. The correlation we found between weight and pNfL concentration was negative. A positive correlation would be expected if obesity and associated peripheral neuropathy was the underlying mechanism driving this association.

We did not find an association between pNfL concentration and sex, which is in agreement with earlier work [7]. The relationship between sex and progression of human neurodegenerative disease is not well-defined, although recent work indicates sex affects the relationship between BMI and cognitive aging in humans [22]. The effect of neuter status on pNfL concentration could not be robustly evaluated in this study due to low numbers of intact dogs. However, it should be considered in future work as sex hormones could be influential to NfL concentration in the dog model.

In Labrador Retrievers, coat color is associated with a number of disease conditions, likely through positive selection pressure resulting from artificial selection [23,24]. We found no association with pNfL concentration and coat color in this study population.

Purebred dogs are genetically and phenotypically diverse populations. A single purebred dog population was chosen for this work to mitigate confounding factors associated with this extensive phenotypic and genetic diversity. This approach enabled targeted evaluation of relationships between factors such as height, weight and BMI. However, further work would be necessary to understand how these Labrador specific data relate to other breeds. Given that the trends reported in this Labrador population are also present in humans, we suspect that similar findings will be seen across breeds. Dogs were screened for clinical evidence of neurodegenerative disease using neurologic and physical examination; nerve conduction velocities or advanced imaging were not used as these require sedation and/or general anesthesia in canine patients.

Measurement of pNfL concentration provides a rapid and repeatable sample collection method to monitor for neurodegenerative disease progression or response to novel therapies. pNfL concentration has been established as a useful biomarker for a number of neurodegenerative diseases in humans and is starting to be established as an important biomarker for disease states in canine models.

5. Conclusions

We found that in dogs, pNfL concentration increases with aging and decreasing weight and height. The use of a single breed of dog was advantageous to correlate phenotypic measures to baseline pNfL concentration in this population, providing important insight into variables that can affect pNfL concentration in the canine model.

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CRediT authorship contribution statement


Declarations of Competing Interest

Henrik Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Summed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecere and Biogcn, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the EU Ventures Incubator Program (outside submitted work). The other authors declare no competing interests.

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Appendix A. Supplementary data

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