

1 **Analysis of neurofilament concentration in healthy adult horses**
2
3 **and utility in the diagnosis of equine protozoal**
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5 **myeloencephalitis and equine motor neuron disease**
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37 **Running title:** Neurofilaments as biomarker for neurological
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39 disorders
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59 **Abstract:** Neurofilaments (NFs) are structural proteins of neurons
60 that are released in significant quantities in the cerebrospinal fluid
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64 and blood as a result of neuronal degeneration or axonal damage.
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66 Therefore, NFs have potential as biomarkers for neurologic
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68 disorders. Neural degeneration increases with age and has the
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70 potential to confound the utility of NFs as biomarkers in the
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72 diagnosis of neurologic disorders. We investigated this relationship
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74 in horses with and without neurological diagnosis. While controlling
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76 for horse type (draft, pleasure, and racing), we evaluated the
77
78 relationship between serum heavy-chain phosphorylated
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80 neurofilaments (pNF-H) and age, sex, and serum vitamin E
81
82 concentrations. Serum pNF-H concentrations increased by 0.002
83
84 ng/mL for each year increase in age. There were significant
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86 differences in the serum pNF-H concentration among the type of
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88 activity performed by the horse. The highest serum pNF-H
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90 concentration was found in horses performing heavy work activity
91
92 (racehorse) and with lower serum pNF-H concentration found
93
94 among light (pleasure riding) and moderate (draft) activity. There
95
96 was no significant association between the pNF-H concentration and
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98 sex or vitamin E concentration. Serum pNF-H concentration was
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100 elevated among horses afflicted with EMND and EPM when
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102 compared with control horses without evidence of neurologic
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104 disorders. Accordingly, serum pNF-H concentration can serve as a
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42 useful biomarker to complement the existing diagnostic work-up of
43 horses suspected of having EPM or EMND.

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45 **Key words:** Biomarker; equine motor neuron disease; equine
46 protozoal myeloencephalitis; neurofilaments.

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171 **66 1. Introduction**
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173 67 Current diagnostic methods used to diagnose equine
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175 68 neurologic disorders such as equine protozoal myeloencephalitis
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177 69 (EPM) and equine motor neuron disease (EMND) are based heavily
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179 70 on clinical examinations and invasive laboratory tests, i.e., tissue
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181 71 biopsies and cerebrospinal centesis, and definitive diagnosis can
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183 72 only be determined by autopsy (Dubey et al., 2001; Reed et al.,
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185 73 2013).

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187
188 74 Neurofilaments (NFs) are structural proteins of neurons that
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190 75 are densely located in axons of the neurons (Boylan et al., 2009;
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192 76 Gresle et al., 2014) and mainly consist of 3 subclasses: light (NF-L),
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194 77 medium (NF-M), and heavy (NF-H) (Gresle et al. 2014). These
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196 78 proteins are released into the cerebrospinal fluid (CSF) and blood as
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198 79 a result of neuronal degeneration or axonal damage (Inoue et al.,
199
200 80 2017; Petzold, 2005). Neurofilaments have been demonstrated to be
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202 81 stable in blood, serum, or CSF over time, and no effect has been
203
204 82 detected in pNF-H concentrations when freezing CSF or serum
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206 83 (Gendron et al., 2017; Hamishehkar et al., 2016). The fact that NFs
207
208 84 have a relatively long half-life makes the concentration of NFs in
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210 85 serum a possible biomarker for neurologic diseases or neuronal
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212 86 disintegration (Millecamps et al., 2007; Yuan et al., 2009).

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215 87 Our studies and those of others have confirmed elevated
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217 88 concentrations of neurofilaments in animals affected with several
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227 89 pathologic conditions or trauma (Intan-Shameha et al., 2017;
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229 90 Nishida et al., 2014). Little is known about the changes in the
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231 91 concentrations of NFs during the normal aging process. Our
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233 92 suspicion is that the concentrations of the phosphorylated NFheavy
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235 93 (pNF-H) in the serum increases with age as a result of progressive
236
237 94 neuronal loss and axonal disintegration. The literature on the
238
239 95 relationship between age and neuronal degeneration is conflicting.
240
241 96 Although one study in humans reported that the serum concentration
242
243 97 of phosphorylated NF-light (pNF-L) increases with age (Burianová
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245 98 et al., 2015); studies on the relationship between the serum
246
247 99 concentration of NFs and age are lacking in horses. A study in rats
248
249 100 demonstrated non-significant changes in the number of neurons with
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251 101 age (Vågberg et al., 2015). If the serum concentration of NFs
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253 102 increases significantly with age in the horse, this finding has the
254
255 103 potential to confound the utility of NFs as a test for equine
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257 104 neurologic disorders. Furthermore, in a previous study we reported
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259 105 that horses afflicted with EPM had significantly increased
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261 106 concentrations of pNF-H compared to healthy horses. Since we did
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263 107 not account for the age of the animals in that report (Intan-Shameha
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265 108 et al. 2017), we conducted this study to determine if there was a
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267 109 relationship between pNF-H concentrations and age.
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272 110 Another factor that has the potential to influence the
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274 111 concentrations of NFs in animals is treatment with vitamin E.
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283 112 Vitamin E is commonly administered to animals and humans for
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285 113 disease prevention or treatment of neurodegenerative diseases,
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287 114 including EMND and motor neuron diseases in other species
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289 115 (Brown et al., 2017; Finno et al., 2017; Mohammed Dr. et al., 2012;
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291 116 Ng et al., 2017). The impact of serum concentrations of vitamin E
292
293 117 on the concentration of NFs is not clear and has a potential to
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295 118 confound the utility of this biomarker as a diagnostic parameter for
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297 119 neurologic disorders.

300 120 In this study we aim to investigate the association between
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302 121 pNF-H and the age of the horse, while controlling for other possible
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304 122 confounding factors, (effect of serum levels of vitamin E, level of
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306 123 activity of the horse, and sex) in order to evaluate the potential use
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308 124 of these proteins as biomarkers for 2 common neurologic disorders
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310 125 of the horse: EMND and EPM.

313 126 **2. Materials and methods**

315 127 *2.1. Study design, target and study population*

317 128 This cross-sectional study included horses residing in New
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319 129 York State. The study population included healthy horses and horses
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321 130 with a confirmed neurologic diagnosis of EMND or EPM. The case
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323 131 horses were either admitted to the Cornell University Hospital for
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325 132 Animals (CUHA) or had blood samples submitted to the Animal
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327 133 Health Diagnostic Center (AHDC). Control horses included horses
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329 134 admitted to CUHA for evaluation and treatment of non-neurologic
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339 135 conditions or horses without reported neurologic clinical signs
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341 136 whose blood was submitted to the AHDC for determination of
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343 137 Vitamin E levels. Horses with or without neurologic disorders were
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345 138 categorized into 3 activity categories: light (pleasure or trail riding
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347 139 horses), moderate (working draft horses or low-level event horses)
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350 140 and heavy (Thoroughbred race horses).
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352 141 *2.2 Inclusion criteria*

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354 142 The inclusion criteria for enrollment of case horses with
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356 143 neurological diseases was a definitive diagnosis of EMND or EPM
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358 144 (based on necropsy findings that included histopathological
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360 145 examination of the brain and spinal cord and vitamin E values in
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362 146 their medical record. All horses without a neurologic disorder (lack
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364 147 of reporting of neurologic signs) were included as healthy horses.
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367 148 *2.3 Determination of serum pNF-H concentration*

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369 149 The pNF-H assay was conducted using the pNF-H sandwich
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371 150 enzyme-linked immunosorbent assay (ELISA) kit (ELISA, EMD
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373 151 Millipore, Billerica, MA). The protocol used chicken polyclonal
374
375 152 antibodies generated against pNF-H, which were pre-coated onto a
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377 153 96-well plate, later rabbit polyclonal antibodies and a goat anti-
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379 154 rabbit alkaline phosphatase conjugate were used to detect the
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381 155 captured pNF-H from the samples.
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384 156 The pNF-H ELISA kit uses antibodies specific for pNF-H
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386 157 from mammalian species, additional ELISA protocol details are
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395 158 described previously (Anderson et al., 2008; Intan-Shameha et al.,
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397 159 2017). Frozen serum samples from each horse were thawed prior to
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399 160 assay. All samples were tested in duplicate and the assay was
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401 161 performed according to the manufacturer's protocol. The person
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403 162 who performed the assays was completely blinded to the clinical
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405 163 information. The mean absorbance of the pNF-H standard,
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407 164 measured as optical density (OD), was plotted on a logarithmic
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409 165 scale. As a result, a standard curve was created and was used to
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411 166 calculate the pNF-H concentration of each sample (range of
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413 167 detection was 0.0293 ng/mL to 15 ng/mL), since duplicates were
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415 168 used for every sample, an average value of each sample was
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417 169 calculated.

420 170 *2.4 Determination of vitamin E concentration*

421 171 Serum was harvested from the horse whole blood samples,
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423 172 and 1 mL of serum was added into a sterile polypropylene
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425 173 microtubes containing an antioxidant mixture, consisting of 100 mL
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427 174 of an ethanol mixture of propyl gallate and EDTA. The samples
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429 175 were then frozen at -75°C until testing. Serum concentrations of α -
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431 176 tocopherol were measured at the AHDC at Cornell University by
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433 177 means of high-performance liquid-liquid partition chromatography.
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435 178 Analytes of interest were detected by spectrophotometry (molecular
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437 179 fluorescence emission at 330 nm for 7.05 minutes) with a tandem
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439 180 arrangement of a variable-wavelength UV detector and a
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451 181 spectrofluorometric detector. The concentration of vitamin E was
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453 182 reported as $\mu\text{g/ml}$. Further details on method are delineated
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456 183 previously (Mohammed et al., 2007).

457 458 184 *2.5 Data collection and analysis*

459
460 185 Data on the age, sex and type of activity (draft, pleasure, or
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462 186 racing; which recoded into light, moderate, or heavy) of the horse
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464 187 was acquired from the CUHA horses' medical records or collected
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466 188 by personal interviews with the horse's owner/trainer.

468 189 The data was initially reported using frequency distribution
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470 190 and graphics, and the measure of central tendency (mean or median)
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473 191 and dispersion (standard deviation and range) were calculated. The
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475 192 bivariate relationship between each of the factors/variables (age,
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477 193 vitamin E concentrations and type of horse activity) and the
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479 194 concentration of pNF-H was assessed using regression analysis or
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481 195 analysis of variance for categorical variables. In the final analysis,
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483 196 factors that were significantly associated with the concentrations of
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485 197 pNF-H in the bivariate analysis were evaluated jointly using the
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487 198 general linear model to assess the significance of association of each
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489 199 factor while simultaneously controlling for other factors. The
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491 200 probability of neurologic diseases (EMND or EPM) was calculated
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493 201 from the logistic regression analysis equation and the dependent
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495 202 variable was whether the horse had neurologic disorder or not. Only
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498 203 horses with vitamin E values were included in this analysis. All
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507 204 statistical analyses were performed using the SPSS v.24 (IBM SPSS
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509 205 Statistical Software, White Plain, NY) and statistical significance
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511 206 differences were considered a type I error (*p-value*) of 0.05.

513 207 **3. Results**

514 208 *3.1 pNF-H as a function of age, sex and activity*

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516 209 A total of 169 horses without clinical signs of neurologic
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518 210 disease met the inclusion criteria and were enrolled for this part of
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520 211 the study. **Table 1** shows the distribution of the pNF-H horse serum
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522 212 concentrations by the type of activity, sex and age of the horse.
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524 213 There was significant variation in the serum neurofilament
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526 214 concentrations among horses with different levels of work activity
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528 215 (light, moderate, and heavy). Those horses with heavy activity
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530 216 (Thoroughbred race horses), had significantly higher serum levels
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532 217 of neurofilaments compared to either horses with moderate work
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534 218 activity (working draft horses or low-level event horses) or light
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536 219 work activity (pleasure horses or trail horses) (**Table 1**). There was
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538 220 no significant difference in the serum concentration of
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540 221 neurofilaments among horses in our study population based upon
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542 222 the sex of the horse.

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544 223 The average age of the healthy horses in our study was 11.65
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546 224 years (SD = 6.8 years) (**Table 1**). There was a tendency for the
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548 225 concentrations of pNF-H to increase with the age of the horse
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550 226 (**Figure 1**). The initial correlation (bivariate) between age and the
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564 227 level of neurofilaments was evaluated using a regression analysis
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566 228 with different transformation (linear and second order) to ensure
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568 229 capturing any variability in age. Although there was significant
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570 230 positive linear relationship between the age of the horses in this
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572 231 study and the concentrations of the pNF-H; the concentrations of
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574 232 pNF-H increased by only 0.002 ng/ml for each year of increase in
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576 233 age.

578 234 *3.2 pNF-H concentration and vitamin E concentration*

580 235 Serum vitamin E concentrations were obtained from 93
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582 236 healthy horses. The average serum vitamin E concentration in
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584 237 healthy horses was 2.56 µg/ml (**Table 1**). There was no significant
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586 238 correlation between the concentration of vitamin E and the
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588 239 concentration of pNF-H in our study (**Figure 2**) and in the bivariate
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590 240 analysis.

593 241 *3.3 Multivariate Analysis*

595 242 **Table 2** shows the results of the multivariate analysis for the
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597 243 relationship between the age of the horses and the serum
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599 244 concentrations of pNF-H when controlled for the activity of the
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601 245 horses. The concentrations of neurofilaments increased by 0.002
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603 246 ng/ml for each year of increase in age of the horse (regression
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605 247 coefficient). That means for each year increase in age of the horse
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607 248 the concentration of pNF-H increases by 0.002 What that means
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619 249 There was a significant association between the type of
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621 250 activity of the horse and the concentrations of pNF-H. The adjusted
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623 251 mean pNF-H values for the reference category of the activity was 0
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625 252 ng/ml, horses with heavy activity was 0.359 ng/ml which was
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627 253 significantly higher than that for moderate (0.225 ng/mL) or light
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629 254 (0.0246 ng/mL) activity horses (**Table 2**).

630 255 *3.4 pNF-H and neurologic disorders*

634 256 We investigated the association between serum pNF-H
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636 257 concentration and the likelihood of neurologic disorders using a
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638 258 logistic regression analysis. A total of 61 horses with confirmed
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640 259 diagnosis of EMND (23 horses) or EPM (38 horses) were identified.
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642 260 The probability of neurologic disorder given the concentrations of
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644 261 neurofilaments was calculated using the logistic regression analysis
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646 262 as follows: $P(\text{Neurologic}) = \frac{1}{1 + (\exp^{-(\alpha + \beta(pNFH))})}$. Where P
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648 263 (Neurologic) is the probability of neurologic disorders (EMND or
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650 264 EPM), α is the constant of the logistic regression, and β is the
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652 265 regression coefficient for the changes in the probability of pNF-H
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654 266 per unit change in the pNF-H concentrations. In the analysis the
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656 267 constant value was -3.786 and the regression coefficient was 2.977.
657
658 268 **Figure 3** shows the relationship between the probability of
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660 269 neurologic disorder and the pNF-H serum values. The probability of
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662 270 a neurologic disorder reaches 0.9 as the concentrations of pNF-H
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664 271 reaches 2.0 ng/ml (**Figure 3**).

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675 **272 4. Discussion**
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677 273 The long-term objective of our research is to investigate the
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679 274 usefulness of pNF-H as a diagnostic parameter for the presence and
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681 275 severity of neurologic disorders in the horse. The use of pNF-H as a
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683 276 diagnostic marker for neurologic disorders, i.e., amyotrophic lateral
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685 277 sclerosis (ALS), or brain injuries in humans, has proven to be useful
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687 278 (Chen et al., 2016; Gaiani et al., 2017; Gendron et al., 2017; Poesen
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689 279 et al., 2017; Shibahashi et al., 2016). Studies have linked the
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691 280 concentrations of these proteins to certain neurologic conditions in
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693 281 humans and in horses (Idland et al., 2017, Takei, 1992). Most of the
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695 282 aforementioned neurologic conditions, in humans or animals, are
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697 283 age related and it is not clear whether the observed association with
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699 284 pNF-H was confounded by the age of the study units—either
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701 285 humans or animals. Hence, it is imperative to investigate whether an
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703 286 association between serum concentrations of pNF-H and the
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705 287 likelihood of neurologic disorders in horses is likely to be
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707 288 confounded by the age of the horse.
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711 289 The potential confounding effect of age is plausible. It is
712
713 290 common knowledge that the neurons degenerate and die with age,
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715 291 so as a consequence it is reasonable to expect a proportionate
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717 292 increase of the concentration of NFs with age. Neurofilaments are
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719 293 found in both the central and peripheral nervous system (Petzold,
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721 294 2015). As a consequence of neuronal or axonal damage associated
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731 295 with the aging process or trauma, NFs are believed to be released
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733 296 into the extracellular space increasing the concentration of pNF-H
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736 297 in the CSF and serum (Petzold, 2015; Steinacker et al., 2016b,
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738 298 2016a). Several studies have used this finding to develop biomarkers
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740 299 for neurodegenerative diseases and traumatic conditions in humans
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742 300 and experimental animals (Intan-Shameha et al., 2017; Kirkcaldie
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744 301 and Collins, 2016; Yilmaz et al., 2017).

746 302 This study showed that although there was a positive change
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748 303 in the relationship between age and serum pNF-H with age, the
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750 304 degree of change was not high. Reports in humans have
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752 305 demonstrated similar association based upon examination of CSF
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754 306 (Bjerke et al., 2014; Steinacker et al., 2016b, 2016a). The difference
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756 307 between our study and the aforementioned studies is that in the
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758 308 human studies the concentrations of pNF-H were measured in the
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760 309 CSF and not serum.

763 310 To the authors' knowledge, this is the first study to
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765 311 investigate the association between serum concentration of NFs and
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767 312 age in the horse. Since CSF and serum pNF-H concentrations have
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769 313 a direct proportional relationship, we looked at previous studies that
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771 314 evaluated the association between serum pNF-H concentrations and
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773 315 age in human patients. Although several studies demonstrated that
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775 316 the concentration of pNF-H in the CSF was associated with age-
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777 317 related neurodegeneration in cognitively healthy adults, other
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787 318 studies were not able to make similar conclusions (Idland et al.,
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789 319 2017; Vågberg et al., 2015). Most of these human studies examined
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791 320 the relationship between age and the CSF-NFs concentrations by
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793 321 assessing the deterioration in the whole-brain (Bjerke et al., 2014;
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795 322 Steinacker et al., 2016b, 2016a; Vågberg et al., 2015; Zetterberg,
796
797 323 2017). The consensus among those studies was that there are age-
798
799 324 related changes in the human brain tissue that reflect the ageing
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801 325 process and that concentration of the NFs measured in the CSF
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803 326 demonstrated high correlation between the NF-L and NF-H.
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805 327 Whereas in two studies the age was biased towards elderly
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807 328 individuals, other studies patients' age range was skewed (Idland et
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809 329 al., 2017; Steinacker et al., 2016a, Takasaki et al., 2002).
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811 330 Interestingly, the human study populations included only mature
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813 331 subjects ranging from 20 to 70 years of age (Vågberg et al., 2015;
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815 332 Zetterberg, 2017). In our study, horse age ranged from 0.58 to 31
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817 333 years of age (11.65 mean) and included juvenile individuals.
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820
821 334 The vitamin E concentrations in serum are known to be
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823 335 associated with aging in several neurologic disorders in animals and
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825 336 humans (Divers et al., 2006; Hamishehkar et al., 2016). The criteria
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827 337 for including horses in the normal category included a cutoff point
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829 338 for vitamin E of concentrations > 1.5 ug/mL. This cut-off point was
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831 339 based both upon our clinical experience and experimental findings
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833 340 (Divers et al., 2006). In the final analysis for assessing the
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843 341 association between age and concentrations of vitamin E, only
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845 342 horses with vitamin E values recorded in the medical record were
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848 343 included in the study. Since there is an association between the NF
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850 344 concentrations and age, it is not unreasonable to hypothesize that the
851
852 345 NF serum concentrations might be associated with serum vitamin E
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854 346 concentration. However, in our study population there was no
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856 347 significant association between serum concentrations of NF and
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858 348 serum vitamin E among the healthy horses. To the authors'
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860 349 knowledge there is no previous study that examined this association
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862 350 in blood samples from human or animals. The only study that
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864 351 indirectly investigated the relationship between vitamin E and pNF-
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866 352 H, did so by examining histopathological changes and concluded
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868 353 that there was no significant association (Takei, 1992). That
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870 354 observation is consistent with the findings of this study.

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872
873 355 Our study demonstrated significant differences in the
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875 356 concentration of pNF-H among horses performing different levels
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877 357 of activity. Horses undergoing heavy exercise (Thoroughbred racing
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879 358 horses) had higher serum pNF-H concentration than horses
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881 359 undergoing light or moderate (draft, pleasure riding, event horses).
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883 360 Although there are no previous studies in animals that demonstrated
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885 361 an association between serum pNF-H concentrations and levels of
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887 362 activity, several studies in human subjects showed that the serum
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889 363 pNF-H concentrations are increased among competitive athletes
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899 364 (Oliver et al., 2016; Shahim et al., 2017). These studies attributed
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901 365 the increase of pNF-H concentrations among performing athletes to
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903 366 increased likelihood of trauma, concussion, or injury. Given the
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905 367 relatively high level of training activity experienced by
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907 368 Thoroughbred racehorses, it is reasonable to suggest that the
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909 369 significantly higher serum levels of pNF-H found in Thoroughbred
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911 370 racehorses in this study may reflect an increased exposure to
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913 371 exercise-related trauma in comparison to pleasure or draft horses.
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916 372 Recent studies have promoted the use of NFs as a diagnostic
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918 373 biomarker for neurologic conditions in animals and humans
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920 374 (Disanto et al., 2017; Intan-Shameha et al., 2017; Nishida et al.,
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922 375 2014; Steinacker et al., 2016b, 2016a; Toedebusch et al., 2017). In
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924 376 our study we explored the potential use of serum pNF-H
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926 377 concentrations to complement clinical observations and
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928 378 conventional diagnostic tests in the diagnosis of EMND and EPM
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930 379 in the horse. A definitive diagnosis for horses afflicted with these
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932 380 conditions requires histopathological examination of the spinal cord
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934 381 to detect pathognomonic lesions (Divers, T.J.; Mohammed, H.O.;
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936 382 Cummings, J.F.; Valentine, B.A.; De Lahunta, A.; Jackson, C.A.;
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938 383 Summers, 1994; Reed et al., 2016). Both conditions affect the
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940 384 neurons in the CNS and associated axons leading to the release of
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942 385 neurofilaments in the serum. This study demonstrated the value of
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944 386 using elevated concentrations of serum pNF-H as a biomarker to
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955 387 predict the probability of the diagnosis of EMND and EPM in
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957 388 neurologic horses.

959 389 Previous studies of the prognostic value of the neurofilament
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961 390 concentrations had proposed positive cut-off points for the diagnosis
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963 391 of the respective neurologic condition (Nishida et al., 2014;
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965 392 Steinacker et al., 2016b, 2016a). Unlike the previous studies, the
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967 393 authors propose the use of a probability approach for the
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969 394 interpretation of neurofilaments concentrations in the diagnosis of
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971 395 EMND or EPM. This approach is based upon two premises: First,
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973 396 both of these neurologic conditions are progressive in nature and
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975 397 may have subclinical and clinical phases in which the serum
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977 398 concentrations of neurofilaments would likely differ. The
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979 399 probability of the disease would be associated with the specific level
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981 400 of the serum pNF-H value for a particular patient. Second, it is
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983 401 envisaged that serum concentrations of neurofilaments would be
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985 402 only one of the parameters a clinician would use in the diagnostic
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987 403 work-up, including medical history and clinical examination to
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989 404 make a specific diagnosis of EMND or EPM.

993 405 Finally, it can be difficult for equine practitioners to make a
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995 406 differential diagnosis between hind leg lameness and neurologic
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997 407 disease, i.e., EPM. The inclusion of serum neurofilament
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999 408 concentrations in the diagnostic work-up of hind leg lameness of
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1001 409 performance horses has the potential to aid the clinician in making
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1011 410 an accurate differential diagnosis between EPM and a hindlimb
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1013 411 orthopedic lameness, thus enabling evidence-based treatment of the
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1015 412 condition.
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1018 413 In conclusion, our results showed that although serum
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1020 414 concentrations of pNF-H increased slightly with the age of the
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1022 415 horse, the degree of this increase was not statistically significant.
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1024 416 Serum pNF-H concentrations were not affected by the concentration
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1026 417 of vitamin E in the serum, nor did they vary with the sex of the horse.
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1028 418 Finally, the serum pNF-H concentration did vary with the activity
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1030 419 of the horse, with horses undergoing heavy activities had
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1032 420 significantly higher pNF-H values in comparison to light and
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1034 421 moderate activities.
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659 **Table 1.** Distribution of serum phosphorylated neurofilament H
660 (pNF-H) concentration among the different activities and factors
661 investigated in healthy control horses (169).

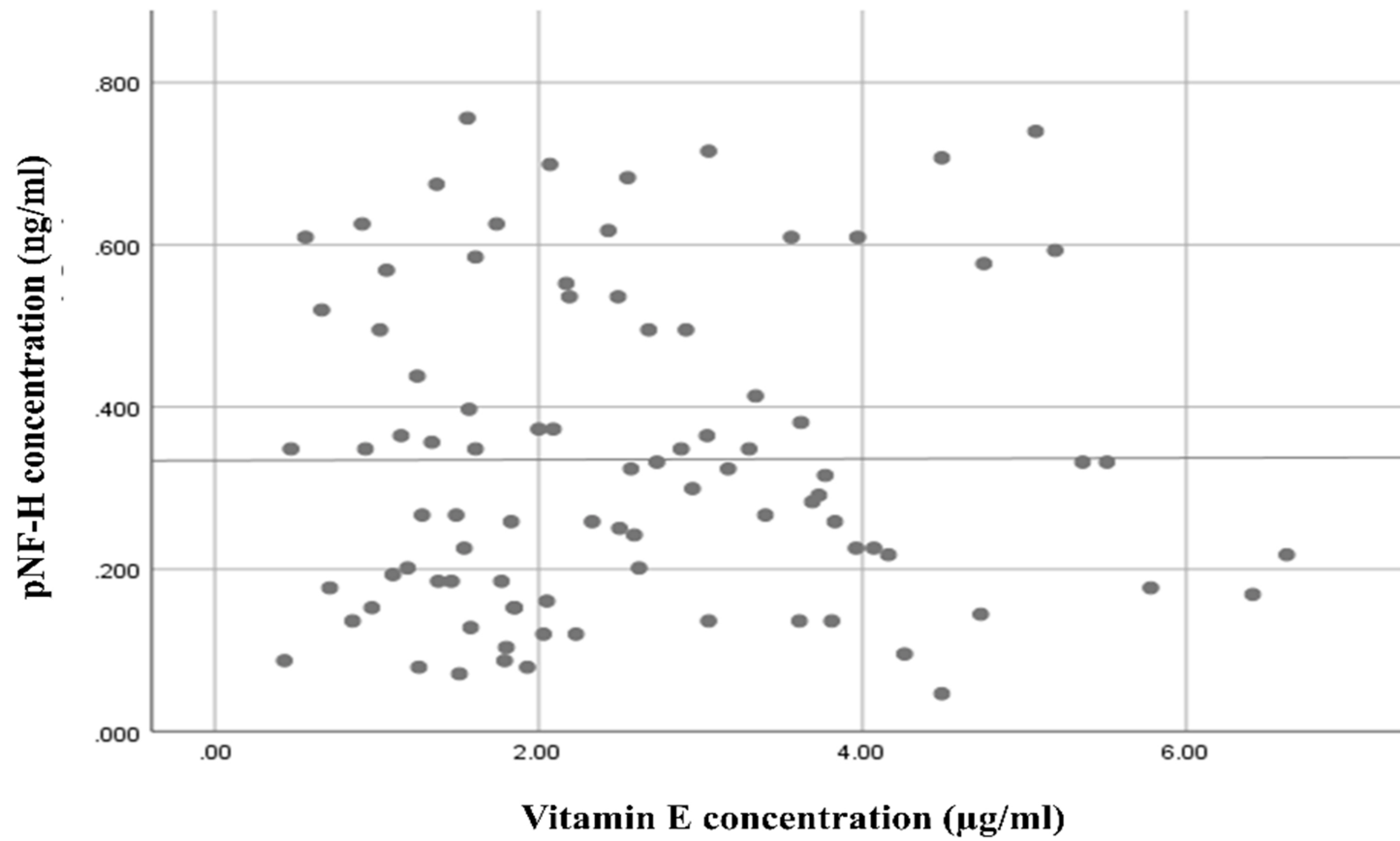
662 **Table 2.** Regression analysis results between the relationship of the
663 horse age and the serum phosphorylated neurofilament H (pNF-H)
664 concentration in healthy control horses.

665 **Figure 1.** The distribution of serum phosphorylated neurofilament
666 H (pNF-H) concentration in horses without neurologic signs and the
667 age of the horse.

668 **Figure 2.** The distribution of serum phosphorylated neurofilament
669 H (pNF-H) in horses without neurologic signs and vitamin E
670 concentration.

671 **Figure 3.** The relationship between serum phosphorylated
672 neurofilament H (pNF-H) concentration and the probability of
673 neurologic disorders (EMND and EPM) as computed in the logistic
674 regression analysis.





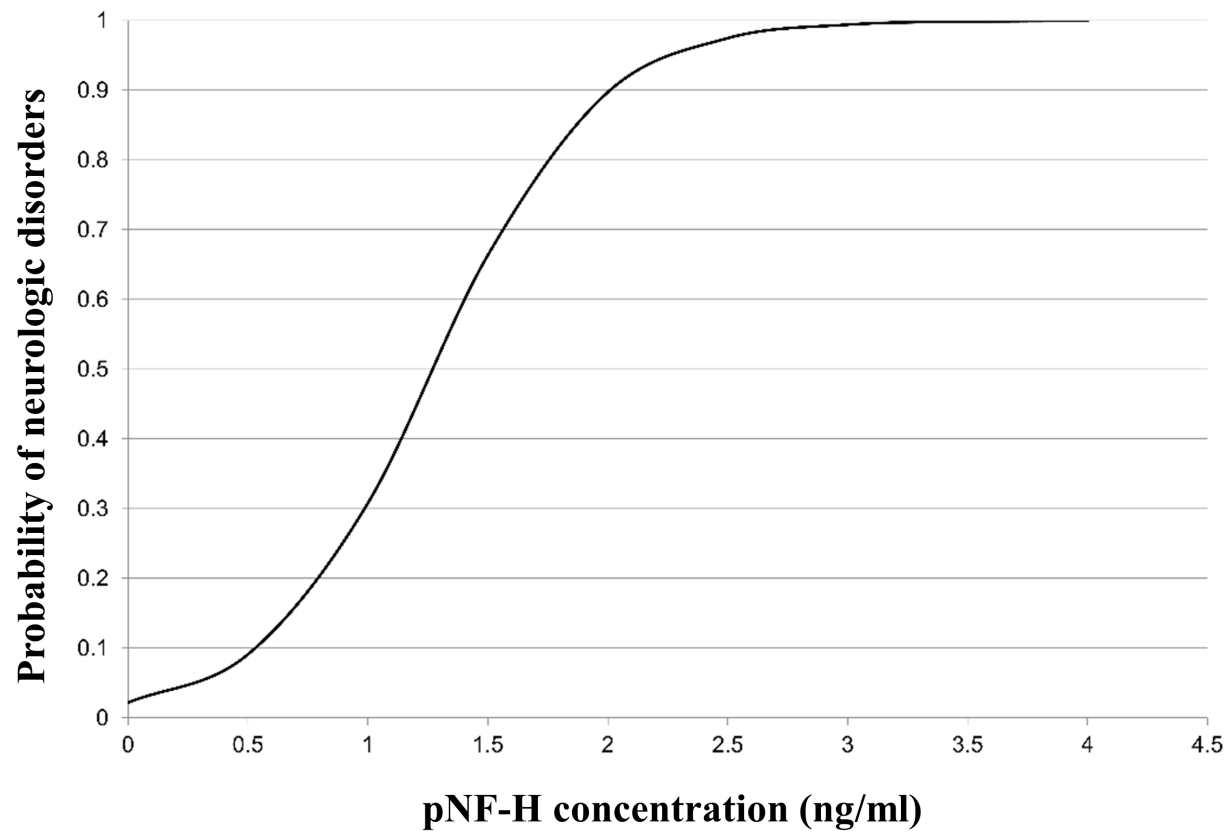


Table 1. Distribution of serum phosphorylated neurofilament H (pNF-H) concentration among the different activities and factors investigated in healthy control horses (169).

Factors	Mean*	Standard error	95% Confidence interval
pNF-H concentration (169)	0.278	0.015	0.248, 0.308
Type of horse activity/level of work			
Light (Pleasure) ^φ (124)	0.270 ^a	0.017	0.236, 0.304
Moderate (Draft) ^ε (27)	0.242 ^a	0.041	0.162, 0.342
Heavy (Thoroughbred) [§] (18)	0.386 ^b	0.015	0.278, 0.494
Sex of the horse			
Mare (71)	0.285	0.023	0.239,0.330
Gelding (89)	0.273	0.022	0.229, 0.317
Intact male (9)	0.271	0.060	0.133, 0.410
Vitamin E (μ/ml) (93)	2.565	1.496	2.268, 2.862
Age (years) (169)	11.65 [¥]	0.52	0.58, 31.0

*: Means with different superscript letter are significantly different from each other

^φ: Pleasure or trail horses

^ε: Working draft horses or low-level eventer

[§]: Racing thoroughbred horses

[¥]: Mean age of the horses in the study

Table 2. Regression analysis results between the relationship of the horse age and the serum phosphorylated neurofilament H (pNF-H) concentration in healthy control horses.

Factor	Regression coefficient	Standard error
Type of horse activity/level of work		
Light (Pleasure) ^φ (124)	-0.130	0.041
Moderate (Draft) ^ε (27)	-0.170	0.050
Heavy (Thoroughbred) [§] (18)	0.0	
Age*	0.002	0.002
Constant	0.359	0.055

*: Age was forced in the model

^φ: Pleasure or trail horses

^ε: Working draft horses or low-level eventer

[§]: Racing thoroughbred horses