Running Title: Neurofilament Light and Mild Brain Injury

Serum Neurofilament Light is Elevated Differentially in Older Adults with Uncomplicated Mild Traumatic Brain Injuries

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Dear Editor,

Please find attached a manuscript entitled "**Serum Neurofilament Light is Elevated Differentially in Older Adults with Uncomplicated Mild Traumatic Brain Injuries**." There is tremendous interest in identifying serum biomarkers that have diagnostic or prognostic value following traumatic brain injury (TBI). However, some biomarkers used in TBI research, such as neurofilament light (NF-L), are elevated in people with neurological or neurodegenerative diseases. As such, it is critical to determine whether these biomarkers are useful for older adults who present with mild TBIs and who have pre-existing neurological conditions. This is the first study to do this. This study has not been previously published, and it has not been submitted to any other journal for peer review. We are hopeful that you will find this manuscript consistent with the objectives of *Brain*, and that you will initiate the peer review process.

Sincerely,

Grant L. Iverson, Ph.D.
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Teemu Luoto, M.D., Ph.D.
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On behalf of our coauthors.

Abstract

There is considerable interest in the use of blood biomarkers as diagnostic screening tests for mild traumatic brain injury (MTBI). Neurofilament light (NF-L) may have diagnostic and prognostic potential as a blood biomarker for MTBI. Elevated NF-L is associated with several neurological disorders associated with older age, however, which could confound its usefulness as a TBI biomarker. We examined whether NF-L is elevated differentially following uncomplicated MTBI in older adults with pre-injury neurological disorders. A sample of 118 adults (mean age=62.3 years, SD=22.5, range=18-100; 52.5% women) presenting to the emergency department (ED) of Tampere University Hospital with an uncomplicated MTBI were enrolled in this study. All had a Glasgow Coma Scale score of 14 or 15. All participants underwent head CT in the ED and showed no macroscopic evidence of injury. Their most common mechanism of injury was a ground-level fall (70.3%). Within 72 hours of injury, blood was collected from participants. The mean time between injury and blood sampling was 8.3 hours (Md=3.5; SD=13.5; IQR=1.9-6.0, range=0.8-67.4, and 90% collected within 19 hours). A sample of 40 orthopedically-injured trauma control subjects recruited from a second ED also were examined. Serum NF-L levels were measured and analyzed using Human Neurology 4-Plex A assay (N4PA) on a HD-1 Single molecule array (Simoa) instrument. A high correlation was found between age and NF-L levels in the total MTBI sample (r=.80), within the subgroups without pre-injury neurological diseases (r=.76) and with pre-injury neurological diseases (r=.68), and in the trauma control subjects (r=.76). Those with MTBIs and pre-injury neurological conditions had higher NF-L levels than those with no pre-injury neurological conditions ($p<.001$, Cohen's $d=1.01$). The subgroup with MTBIs aged ≥60 had higher NF-L levels than those <60 ($p<.001$, $d=1.14$). The subgroup of trauma controls aged 60 or older had

higher NF-L levels than those under the age of 60 ($p<.001$, $d=1.67$). For MTBI patients with no pre-injury neurological conditions, those aged ≥ 60 had higher NF-L levels than those < 60 ($p<.001$, $d=1.18$). For MTBI patients with pre-injury neurological conditions, those aged ≥ 60 had higher NF-L levels than those < 60 ($p<.001$, $d=1.12$). Older age and pre-injury neurological diseases are associated with elevated serum NF-L levels during the first 72 hours following uncomplicated MTBI, which limits the value of this biomarker during this time period. Given the relatively slow temporal profile for the increase in NF-L, and sustained elevation, samples taken at later time points might be more clinically useful in older adults.

Key words: Head Injury; Traumatic Brain Injury; Biomarker; Blood; Neurofilament; Aging; Emergency Treatment

Word Count: 409

Introduction

Axonal damage is a pathological characteristic of traumatic brain injury (TBI), and it has been found to play a role in neuropsychological outcome (Warner *et al.*, 2010). Neurofilaments are intermediate filaments (thread-like structures) (Ishikawa *et al.*, 1968) that are involved in the growth and structure of axons (Eyer and Peterson, 1994). Neurofilament light (NF-L) is the smallest subunit of the neurofilament heteropolymer (a macromolecule composed of several bonded monomer subunits) (Lee *et al.*, 1993). Together with the neurofilament medium and heavy subunits, NF-L is believed to facilitate scaffolding of the neural cytoskeleton—providing structural support for the axon and regulating axon diameter (Heins *et al.*, 1993; Lee *et al.*, 1993). NF-L plays a role in the structure of neurofilaments, assembly of the neurofilament heteropolymer, and formation of a stable stationary neurofilament network (Heins *et al.*, 1993). NF-L is also involved in the transport of neurofilaments through the axon (Yates *et al.*, 2009), axonal and dendritic branching and growth (Eyer and Peterson, 1994), and it is abundant in long subcortical white matter axons (Zetterberg *et al.*, 2013). Proteolytic (i.e., enzyme breakdown) processes that occur during axonal degeneration lead to incomplete neurofilament degradation (Schlaepfer *et al.*, 1984). Therefore, following axonal damage (Schlaepfer *et al.*, 1984) or neuronal degeneration (Disanto *et al.*, 2017), neurofilaments are released and are present in both the cerebrospinal fluid and the bloodstream (Bacioglu *et al.*, 2016; Brureau *et al.*, 2017).

There is steadily growing interest in examining NF-L following neurotrauma. NF-L levels have been found to increase in both cerebrospinal fluid (CSF) and blood during the first week(s) following TBI (Al Nimer *et al.*, 2015; Shahim *et al.*, 2016). They are elevated in those with traumatic structural abnormalities visible on computed tomography (CT) (Korley *et al.*, 2018), highly elevated following severe TBI and diffuse axonal injury (Ljungqvist *et al.*, 2017),

and they are associated with outcome in patients with severe TBIs (Al Nimer *et al.*, 2015; Shahim *et al.*, 2016). NF-L is also elevated following mild neurotrauma, such as sport-related concussion (Shahim *et al.*, 2018). Interestingly, in one study NF-L was reported to increase in American football players, over the course of a season, who had not experienced a symptomatic concussion (Oliver *et al.*, 2016).

There has been a clarion call for more research focusing on TBI in older adults (Gardner *et al.*, 2018). In Western countries, overall hospital admission rates for TBIs are decreasing, while admissions are *increasing* in patients over 65 years (Koskinen and Alaranta, 2008; Perez *et al.*, 2012; Shivaji *et al.*, 2014). Falls are a common cause of ED visits (Hoidrup *et al.*, 2003; Johansen *et al.*, 2011) and TBI (Helling *et al.*, 1999; Sarani *et al.*, 2009; Poyry *et al.*, 2013) in older adults. Because older adults are more likely to have neurological conditions *prior* to brain injury, NF-L might be elevated *differentially* in older adults, compared to middle-aged or younger adults, following mild traumatic brain injury (MTBI). A number of studies indicate that serum NF-L levels are higher in people with diverse neurological and neurodegenerative diseases (Kuhle *et al.*, 2016; Rohrer *et al.*, 2016; Byrne *et al.*, 2017; Disanto *et al.*, 2017; Gattringer *et al.*, 2017; Hansson *et al.*, 2017; Kuhle *et al.*, 2017; Mattsson *et al.*, 2017; Novakova *et al.*, 2017; Weston *et al.*, 2017; De Marchis *et al.*, 2018; Feneberg *et al.*, 2018; Thompson *et al.*, 2018; Wilke *et al.*, 2018). Therefore, as a potential diagnostic or prognostic biomarker for neurotrauma, it is important to determine whether there is an association between age and NF-L levels in individuals who sustain MTBIs. The purpose of this study is to determine whether NF-L is elevated differentially in older adults with pre-existing neurological disorders or diseases who present to the emergency department (ED) following MTBI. We hypothesized that there would be an association between older age and NF-L levels in both orthopedically-injured trauma

control subjects and patients who sustain uncomplicated MTBIs. In addition, we hypothesized that older adults who sustain MTBIs who have pre-existing neurological diseases would have the highest levels of NF-L.

Methods

Participants

A sample of 325 adults and older adults were evaluated in the ED of Tampere University Hospital (Tampere, Finland) following head trauma and enrolled in a study. Of those, 224 underwent head computed tomography (CT) and 190 had no trauma-related abnormalities identified on CT. Of the 190 with normal head CT scans, 130 had their blood sampled for long-term storage. For the final sample, 118 patients were included if their Glasgow Coma Scale score was 14 or 15 in the ED and they had their blood drawn within 72 hours of injury. The mean age of the sample was 62.3 years (SD=22.5, Range=18-100; 52.5% women). Their mechanisms of injury were as follows: ground level fall=70.3%, fall from height or unclassified fall=7.6%, violence=5.9%, sports=5.1%, bicycle accident=3.4%, motor vehicle accident=2.5%, motorcycle accident=0.8%, and other or unknown=4.2%. In the ED, a detailed case report form was completed for each enrolled patient. The percentages of the sample with loss of consciousness documented in the ED records were as follows: yes, witnessed=17.8%, suspected=31.4%, no=41.5%, and unknown=9.3%. The percentages of the sample with post-traumatic amnesia documented in the ED records were as follows: yes=40.7%, no=53.4%, and unknown=5.9%. The percentages with focal neurological signs in the ED were: yes=10.2%, no=88.1%, and unknown=1.7%. The health history of each enrolled patient was reviewed from the electronical patient records. Prior diagnosed diseases (including neurological diseases) were coded according to the ICD-10 classification (see Table 1). All patients provided written informed consent

according to the Declaration of Helsinki. The study protocol was approved by the ethical review board of Pirkanmaa Hospital District, Finland (ethical code: R15045).

Table 1. Pre-injury neurological conditions identified in the centralized electronic medical records.

	% (n)
Mental and behavioral disorders (F01-99)	
Organic, including symptomatic, mental disorders	16.9 (20)
Vascular dementia (F01)	0.8 (1)
Unspecified dementia (F03)	5.9 (7)
Diseases of the nervous system (G00-99)	
Cerebrovascular Disease	16.1 (19)
Nontraumatic Subarachnoid Hemorrhage, *status post*	1.7 (2)
Cerebral Infarction, *status post*	15.3 (18)
Extrapyramidal and movement disorders (G20-26)	3.4 (4)
Other degenerative diseases of the nervous system (G30-32)	5.9 (7)
Alzheimer disease (G30)	5.9 (7)
Episodic and paroxysmal disorders (G40-47)	29.7 (35)
Epilepsy (G40)	3.4 (4)
Transient cerebral ischemic attacks and related syndromes (G45)	8.5 (10)
Nerve, nerve root and plexus disorders (G50-59)	2.5 (3)
Polyneuropathies and other disorders of the peripheral nervous system (G60-64)	1.7 (2)
Diseases of myoneural junction and muscle (G70-73)	1.7 (2)
Cerebral palsy and other paralytic syndromes (G80-83)	0.8 (1)
Other disorders of the nervous system (G90-99)	0.8 (1)
Neoplasms (C00-D48)	
Benign neoplasm of brain and other parts of central nervous system (D33)	0.8 (1)

Note: N=118. Some subjects have more than one diagnosis.

An orthopedically-injured control sample (N=40) was obtained from the ED of Turku University Hospital (Finland) and included a subgroup of a previously published sample (Posti *et al.*, 2017). The orthopedic control sample included 22 men (55%) and 18 women. Their mean age was 52.15 years old (SD=18.83, IQR=35.75-64.75, Range=22-90). The orthopedic injuries sustained by the control sample were as follows (n, percentage of sample): simple ankle fracture (11, 27.5%), complex ankle fracture (10, 25.0%), wrist fracture (5, 12.5%), hip fracture (3, 7.5%), humerus fracture (3, 7.5), and forearm fracture (2, 5.0). One person in the sample (2.5%) sustained each of the following injuries: clavicle fracture, complex pelvic fracture, knee fracture, hand fracture, and foot fracture. We were not able to determine whether some subjects experienced some degree of peripheral nerve injury. Control subjects were not included if there was any suspicion of an acute TBI (injury signs to the head, any suspicion of TBI signs at the time of injury, or symptoms suggesting a possible TBI) or they experienced polytrauma needing intensive care. After completion of the study, the health history of each enrolled patient was reviewed from the electronical patient records. One subject was identified as having cerebrovascular disease and one subject was identified as having Alzheimer's disease. Many of the control subjects underwent an MRI of their brain for research purposes (i.e., n=31, 77.5% of the sample). If on MRI they had clear evidence of small vessel ischemic disease, multiple white matter hyperintensities, small infarcts, or frontal cortical dysplasia (or possible low-grade glioma; n=1) they were classified as have a pre-injury neurological disease (n=14). Some incidental findings on MRI were classified as broadly normal, such as frontal calcification in the corpus callosum (n=1) and venous angiomas or cavernoma (n=3). The one subject with Alzheimer's disease documented in medical records did not undergo an MRI scan for research purposes. The control subjects were divided into three subgroups: no documented pre-injury

neurological disease and broadly normal brain MRI scan (n=16), no documented neurological disease and missing MRI scan (i.e., unknown pre-injury neurological disease; n=9), and those with pre-injury neurological disease (n=15). The study protocol was approved by the ethical review board of the Hospital District of South-West Finland (code: 68/180/2011).

Blood Sampling and Analytics

Venous blood samples for the MTBI group were collected in the ED. The mean time between injury and blood sampling was 8.3 hours (Md=3.5; SD=13.5; IQR=1.9-6.0, range=0.8-67.4, and 90% collected within 19 hours). For the trauma control sample, venous blood samples were collected on the day of injury or the following day. Serum NF-L levels were measured using the Human Neurology 4-Plex A assay (N4PA) on an HD-1 Single molecule array (Simoa) instrument according to instructions from the manufacturer (Quanterix, Lexington, MA). The measurements were performed by board-certified laboratory technicians who were blinded to clinical data. The limit of detection for NF-L was 0.104 pg/mL and the limit of quantification was 0.241 pg/mL with a calibration range of 0.533 to 453 pg/mL. Two internal quality control (QC) samples were analyzed in each run. For a QC sample with a concentration of 13.9 pg/mL, repeatability and intermediate precision was 4.4%, while for a QC sample with a concentration of 7.1 pg/mL, repeatability and intermediate precision was 6.1%. For the trauma control sample with a concentration of 13.9 pg/mL, repeatability and intermediate precision was ___%, while for the sample with a concentration of 7.1 pg/mL, repeatability and intermediate precision was ___%. The trauma control sample was run at a different time than the MTBI sample, and the two runs were not calibrated for each other.

Statistical Analyses

The associations between age and NF-L were examined in each group separately using Spearman correlations and nonparametric interferential statistics (Mann Whitney U tests) because both the biomarker levels and age were not normally distributed. Nonparametric analyses were also used to examine subgroups with the MTBI group with and without pre-injury neurological diseases. Receiver operator characteristic curve (ROC) analyses with a nonparametric estimate of the area under the curve were used to compare patients with MTBIs to trauma control subjects.

Results

Descriptive statistics for NF-L for the groups and subgroups are presented in Table 2. Within the orthopedically-injured trauma control group, the Spearman correlation between age and NF-L values was high (r=.76). The subgroup aged 60 or older had higher NF-L levels than those under the age of 60 (U=349, p<.001, Cohen's d=1.67; also see Figure 1). There was one extreme outlier in the trauma control group (NF-L level of 140 pg/mL), a man in his mid-late 60s who was later found to have multiple periventricular white matter changes on brain MRI. The subgroup of control subjects with neurological disorders was older than the subgroup without neurological disorders (U=229, p<.001, d=2.37), and they also had higher NF-L levels (U=184.5, p=.011, d=1.59).

Within the uncomplicated MTBI group, there was no significant difference in NF-L levels between men and women (U=1,470, p=.152). The Spearman correlation between age and NF-L values was high (r=.80). The correlations between age and NF-L in subgroups of those with no pre-injury neurological conditions (r=.76) and pre-injury neurological conditions (r=.68) were high. Those with pre-injury neurological conditions had higher NF-L levels than those with no pre-injury neurological conditions (Md=30.82 vs. 12.64, respectively; U=2,638, p<.001,

d=1.01). The subgroup aged 60 or older had higher NF-L levels than those under the age of 60 (Md=28.16 vs. 7.52; U=3,048, p<.001, d=1.14). For those with no pre-injury neurological conditions, those age ≥ 60 had higher NF-L levels than those age < 60 (Md=19.80 vs. 7.15; U=850, p<.001, d=1.18). For those with pre-injury neurological conditions, those age ≥ 60 had higher NF-L levels than those age < 60 (Md=35.20 vs. 8.36; U=404, p<.001, d=1.12).

A ROC analysis under a nonparametric assumption revealed a modest statistically significant area under the curve (AUC) estimate of 0.635 (SE=0.48, p<.011, 95% CI=0.540-0.730) for differentiating those with uncomplicated MTBIs from the trauma control subjects. However, when those with known pre-injury neurological diseases were excluded from the MTBI group only, there was no significant differentiation between the groups (AUC=0.524, SE=0.058, p=.684, 95% CI=0.409-0.638). When those with known pre-injury neurological diseases were excluded from both groups, there was no significant differentiation between the groups (AUC=.601, SE=.065, p=.139, 95% CI=0.474-0.729), despite the fact that the MTBI group (n=64) was older (M=54.5 years, SD=23.1) than the control subjects (n=25; M=43.7 years, SD=16.3). There was no significant differentiation between age-stratified subgroups with uncomplicated MTBIs and the trauma control subjects who were under the age of 60 (AUC=0.498, SE=0.071, p=981, 95% CI=0.360-0.637) or over the age of 60 (AUC=0.582, SE=0.079, p=.317, 95% CI=0.427-0.736). There was a significant differentiation between subgroups with uncomplicated MTBIs and the trauma control subjects, both of whom had pre-injury neurological disease (AUC=.678, SE=.076, p=.036, 95% CI=0.528-0.827).

Table 2. Descriptive statistics for NF-L for the groups and subgroups.

Sample	N	M	Md	SD	IQR	Range
Total Orthopedically-Injured Control Sample	40	18.30	10.65	23.35	6.83-20.68	4.10-141.30
Women	18	11.81	9.55	10.53	6.48-12.03	4.10-51.20
Men	22	23.60	13.15	29.28	7.90-26.90	4.60-141.30
Age < 60	25	8.94	8.20	4.20	5.80-11.20	4.10-23.50
Age ≥ 60	15	33.89	24.40	32.74	16.70-44.70	9.80-141.30
No Pre-Injury Neurological Conditions*	16	9.41	7.55	4.99	5.65-13.05	4.10-23.50
Unknown Pre-Injury Neurological Conditions*	9	19.43	10.30	17.20	8.95-35.10	5.40-51.20
Pre-Injury Neurological Conditions*	15	27.09	17.60	33.91	9.80-31.10	4.60-141.30
Total Uncomplicated MTBI Sample	118	32.07	18.97	40.43	9.05-35.40	2.62-246.92
Men	56	28.79	15.05	40.33	7.52-31.11	2.62-237.30
Women	62	35.04	20.88	40.62	10.29-47.06	3.40-246.92
Age < 60	46	10.33	7.52	7.80	5.54-11.49	2.62-36.44
Age ≥ 60	72	45.96	28.16	46.40	18.42-51.01	9.06-246.92
No Pre-Injury Neurological Conditions	64	17.16	12.64	14.93	7.04-21.60	2.62-85.44
Pre-Injury Neurological Conditions	54	49.75	30.82	52.50	16.96-68.47	3.40-246.92
Age < 60 No Pre-Injury Neurological Conditions	37	11.00	7.15	8.49	5.51-13.08	2.62-36.44
Age < 60 Pre-Injury Neurological Conditions	9	7.58	8.36	2.73	4.89-9.56	3.40-11.69
Age ≥ 60 No Pre-Injury Neurological Conditions	27	25.60	19.80	17.67	14.84-31.92	9.06-85.44
Age ≥ 60 Pre-Injury Neurological Conditions	45	58.18	35.20	53.69	23.18-86.21	10.11-246.92

Note: *The average ages for control subjects were as follows: No Neurological Conditions=M=36.2, Md=33.5, SD=11.5; Neurological Conditions Unknown=M=57.1, Md=55.0, SD=15.4; and Pre-Injury Neurological Conditions=M=66.2, Md=65.0, SD=13.9.

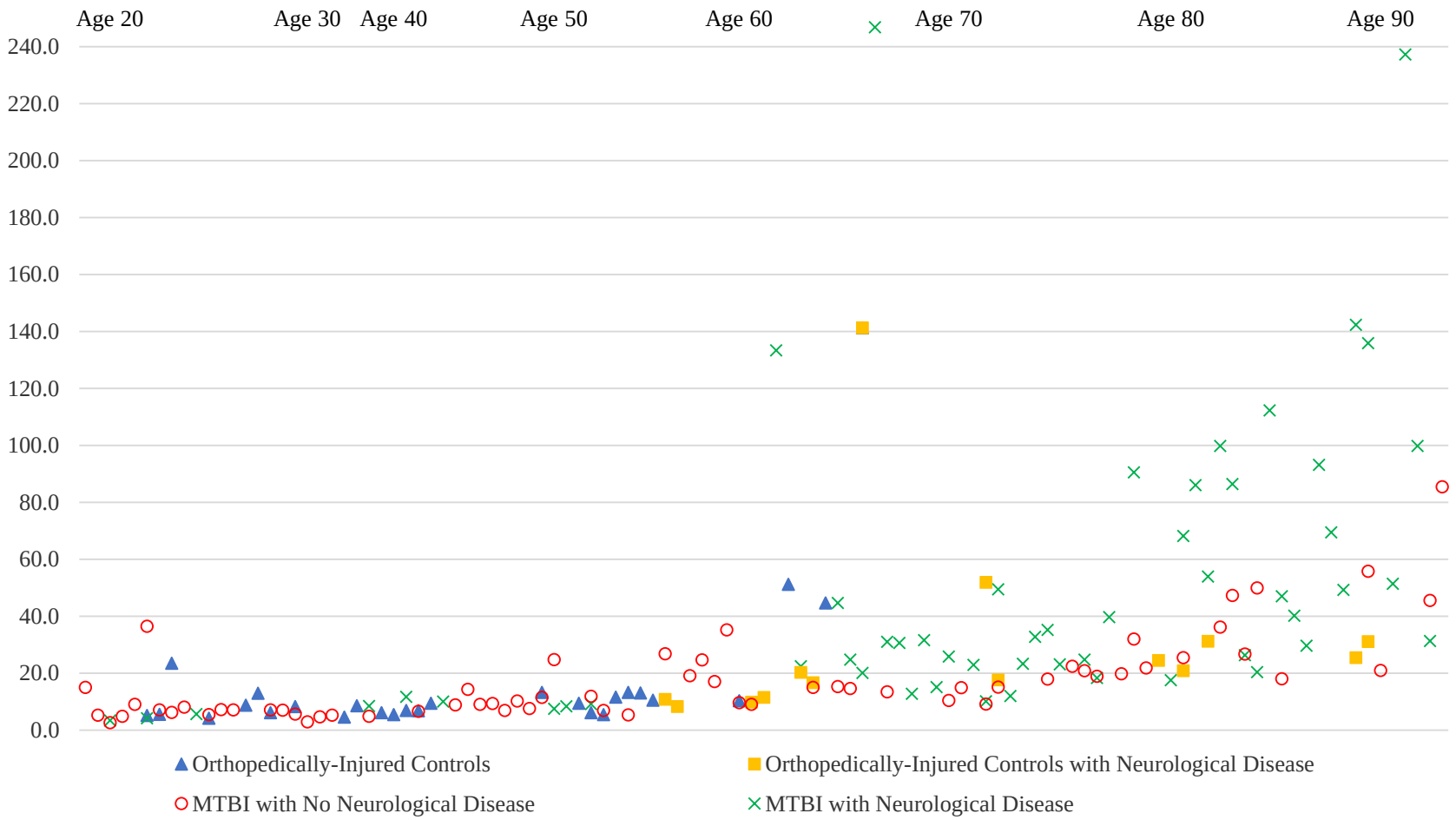
Figure 1 illustrates every individual subjects' serum NF-L level. The subjects are sorted by age, beginning with age 18 and ending with age 100. Notice that serum NF-L levels are well below 20 for the large majority of people in all three groups under the age of 60. As seen in

Table 2, 75% of those with MTBIs who are under the age of 60 have NF-L values less than 11.5 (see the IQR). In contrast, individuals in their 70s, 80s, and 90s have highly elevated NF-L levels compared to young and middle-aged adults. Older adults with pre-injury neurological conditions, in general, have higher NF-L levels than older adults who do not have pre-injury neurological conditions (see Table 2 and Figure 1).

Figure 1. Neurofilament light levels in adults and older adults sorted by age. (Original graph)

Note: Serum NF-L levels are presented for each individual subject, sorted by age, beginning with age 18 and ending with age 100. In all three groups, adults who are 60 or older have significantly higher NF-L levels than adults who are under the age of 60. Y-axis: NF-L concentrations (pg/mL)

Figure 1. Neurofilament light levels in adults and older adults sorted by age. (New Graph)



Note: Serum NF-L levels are presented for each individual subject, sorted by age, beginning with age 18 and ending with age 100. Y-axis: NF-L concentrations (pg/mL). The patients with MTBIs who had neurological diseases had those diseases documented in their medical records. In contrast, the orthopedically-injured control subjects were classified as having neurological diseases if they had an MRI for research purposes and that MRI showed evidence of white matter ischemic disease and/or microinfarcts.

Discussion

There is considerable interest in understanding the diagnostic and prognostic potential of NF-L as a serum biomarker for axonal injury associated with TBI (Al Nimer *et al.*, 2015; Shahim *et al.*, 2016; Korley *et al.*, 2018). It is recognized that TBI in older adults is understudied (Gardner *et al.*, 2018), and the effects of neurotrauma on the aging brain are not well understood. It is essential to examine NF-L in association with aging and neurological diseases because these factors might fundamentally compromise its usefulness as biomarker for TBI in older adults. Our study is the first to examine whether NF-L is elevated differentially following uncomplicated MTBI in older adults with pre-existing neurological disorders. As hypothesized, we found that age is associated with NF-L levels in subgroups of individuals with MTBIs and no pre-injury neurological disorders and in those with pre-injury neurological disorders. Specifically, individuals age ≥ 60 with MTBIs had higher NF-L levels than individuals under the age of 60 for those with pre-injury neurological disorders and for those without pre-injury neurological disorders. We also found that, overall, individuals with MTBIs and pre-injury neurological disorders had higher NF-L levels than individuals without pre-injury neurological disorders. The strong association between older age and higher NF-L levels was also present in the orthopedically-injured trauma control sample. In general, NF-L was not particularly useful for differentiating those with uncomplicated MTBIs from trauma control subjects.

Our results are consistent with past studies showing that serum levels of NF-L are higher in people with diverse neurological and neurodegenerative conditions. Researchers have reported that serum levels of NF-L are higher in people with multiple sclerosis (Kuhle *et al.*, 2016; Disanto *et al.*, 2017; Kuhle *et al.*, 2017; Novakova *et al.*, 2017), acute ischemic stroke (De Marchis *et al.*, 2018), active cerebral small vessel disease (Gattringer *et al.*, 2017), familial

Alzheimer's disease (Mattsson *et al.*, 2017; Weston *et al.*, 2017), Huntington's disease (Byrne *et al.*, 2017), frontotemporal dementia (Rohrer *et al.*, 2016), Creutzfeldt-Jakob disease (Thompson *et al.*, 2018), Parkinsonian disorders (Hansson *et al.*, 2017), other degenerative ataxias such as multiple system atrophy (Wilke *et al.*, 2018) and amyotrophic lateral sclerosis (Feneberg *et al.*, 2018).

Regarding clinical usefulness, there are no established and validated cutoff values for NF-L as a biomarker for TBI, of any severity, across the lifespan, using any commercially-available assay. It is important to note that studies suggest that NF-L has quite slow serum level dynamics. After acute injury it appears to reach its peak concentration more than 7 days after the injury (Shahim *et al.*, 2016; Shahim *et al.*, 2018). NF-L may therefore be of limited use in the Emergency Department but may prove to be a useful biomarker for predicting clinical outcome with samples taken several days following injury (e.g., day 7-10). Future researchers can address the methodological limitations of the present study, and gaps in the literature more broadly, by (i) examining NF-L levels in older adults stratified by brain injury severity (i.e., mild, moderate, and severe); (ii) studying the temporal kinetics of NF-L in the bloodstream after TBI to determine reliable time windows for acute diagnostics of TBI and outcome prediction (across the lifespan); (iii) determining if NF-L levels can differentiate older adults with mild head trauma and positive or negative day-of-injury head computed tomography findings; and (iv) examining if extracerebral and especially peripheral nerve injuries affect the clinical reliability of NF-L in TBI diagnostics. Much additional research is needed to determine whether NF-L is useful for diagnostic or prognostic purposes following TBIs of all severities in older adults.

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Considerations

We mention that these groups were analyzed in separate runs and that the results were not calibrated. However, there is no discussion whether this could influence our results.

Further still, I am not sure if the age should be included in the analysis in other ways than as a correlation. Dividing the patients to those above and below 60 is arbitrary and has no biological basis. Correlation between age and NF-L levels is more informative, together with figure 1. There are many assays in routine clinical use where the normative values are age-adjusted. What comes to TBI biomarkers, NF-L is not an exception, similar problems have been found e.g. with S100B (Fluids Barriers CNS. 2016 Nov 30;13(1):21). I guess we should simply state that if NF-L will be developed for clinical use in TBI, the normative values should probably be age-adjusted and that the results may not be reliable in those with pre-existing neurological diseases.

GI says: visual inspection of the age-ordered data in Figure 1, and the strong correlation between age and NF-L, is very interesting. I agree that it is somewhat arbitrary to choose 60 to create groups.

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