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

Article *in* Journal of Neurotrauma · February 2019

DOI: 10.1089/neu.2018.6341

CITATIONS
8

READS
124

8 authors, including:

Jussi Posti
Turku University Hospital
110 PUBLICATIONS **1,036** CITATIONS

SEE PROFILE

Olli Tenovuo
Turku University Hospital
163 PUBLICATIONS **5,304** CITATIONS

SEE PROFILE

Juha Ohman
Tampere University Hospital (Tays)
210 PUBLICATIONS **5,730** CITATIONS

SEE PROFILE

Teemu Luoto
Tampere University Hospital (TAUH)
159 PUBLICATIONS **1,047** CITATIONS

SEE PROFILE

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

Grant L. Iverson, Ph.D.
Department of Physical Medicine and Rehabilitation, Harvard Medical School; Spaulding Rehabilitation Hospital; & Home Base, A Red Sox Foundation and Massachusetts General Hospital Program, Boston, Massachusetts, USA.
Email: giverson@mgh.harvard.edu
Address: Center for Health and Rehabilitation Research, Department of Physical Medicine & Rehabilitation, Harvard Medical School, 79/96 Thirteenth Street, Charlestown Navy Yard, Charlestown, MA, 02129, USA.

Preethi J. Reddi
Department of Biology, Emory University, Atlanta, GA, USA
Email: preethi.reddi@emory.edu
Address: Department of Biology, 1510 Clifton Rd #2006, Emory University, Atlanta, GA 30322, USA

Jussi P. Posti, M.D., Ph.D.
Division of Clinical Neurosciences, Department of Neurosurgery, and Turku Brain Injury Centre, Turku University Hospital, and University of Turku, Turku, Finland.
Email: jussi.posti@utu.fi
Address: Kiinamyllynkatu 4-8, FI-20521 Turku, Finland

Anna-Kerttu Kotilainen, B.M.
Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland
Email: kotilainen.anna-kerttu.x@student.uta.fi
Address: Faculty of Medicine and Life Sciences, University of Tampere, P.O. Box 100, FI-33014 University of Tampere, Tampere, Finland

Olli Tenovuo, M.D., Ph.D.
Turku Brain Injury Centre, Turku University Hospital and University of Turku, Finland
Email: olli.tenovuo@tyks.fi
Address: Kiinamyllynkatu 4-8, FI-20520 Turku, Finland

Juha Öhman, M.D., Ph.D.
Department of Neurosurgery, Tampere University Hospital and University of Tampere, Tampere, Finland.
Email: juha.ohman@pshp.fi
Address: P.O. Box 2000, Teiskontie 35, FI-33521, Tampere, Finland

Henrik Zetterberg, M.D., Ph.D.
Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
UK Dementia Research Institute at University College London, London, United Kingdom

Department of Neurodegenerative Disease, University College London Institute of Neurology, Queen Square, London, United Kingdom
Email: henrik.zetterberg@clinchem.gu.se
Address: Blå Stråket 15, Vån 3 SU/Sahlgrenska, 413 45 Göteborg, Sweden

Kaj Blennow, M.D., Ph.D.
Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
Email: kaj.blennow@neuro.gu.se
Address: Blå Stråket 15, Vån 3 SU/Sahlgrenska, 413 45 Göteborg, Sweden

Teemu M. Luoto, M.D., Ph.D.*
Department of Neurosurgery, Tampere University Hospital and University of Tampere, Tampere, Finland
Email: teemu.luoto@pshp.fi
Address: P.O. Box 2000, Teiskontie 35, FI-33521, Tampere, Finland

*Corresponding author:
Dr. Teemu M. Luoto
Tampere University Hospital, Department of Neurosurgery
P.O. Box 2000
Teiskontie 35
FI-33521 Tampere, Finland
Email: Teemu.Luoto@pshp.fi; Mobile: +358407039696

Running Title: Neurofilament Light and Mild Brain Injury

Table of Contents Title: Neurofilament Light in Mild TBI

Abstract

Neurofilament light (NF-L) might have diagnostic and prognostic potential as a blood biomarker for mild traumatic brain injury (MTBI). However, elevated NF-L is associated with several neurological disorders associated with older age, 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. In a case-control study, 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) with an uncomplicated MTBI were enrolled. All participants underwent head CT in the ED and showed no macroscopic evidence of injury. 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 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 $d=1.01$). Older age and pre-injury neurological diseases are associated with elevated serum NF-L levels in patients with head trauma and in orthopedically-injured control subjects.

Key words: Traumatic Brain Injury; Biomarker; Blood; Neurofilament; Aging

Introduction

Axonal damage is a pathological characteristic of traumatic brain injury (TBI), and it has been found to play a role in neuropsychological outcome.[1] Neurofilaments are intermediate filaments (thread-like structures)[2] that are involved in the growth and structure of axons.[3] Neurofilament light (NF-L) is the smallest subunit of the neurofilament heteropolymer (a macromolecule composed of several bonded monomer subunits).[4] 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.[4, 5] NF-L plays a role in the structure of neurofilaments, assembly of the neurofilament heteropolymer, and formation of a stable stationary neurofilament network.[5] NF-L is also involved in the transport of neurofilaments through the axon,[6] axonal and dendritic branching and growth,[3] and it is abundant in long subcortical white matter axons.[7] Proteolytic (i.e., enzyme breakdown) processes that occur during axonal degeneration lead to incomplete neurofilament degradation.[8] Therefore, following axonal damage[8] or neuronal degeneration,[9] neurofilaments are released and are present in both the cerebrospinal fluid and the bloodstream.[10, 11]

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.[12, 13] They are elevated in those with traumatic structural abnormalities visible on computed tomography (CT),[14] highly elevated following severe TBI and diffuse axonal injury,[15] and they are associated with outcome in patients with severe TBIs.[12, 13] NF-L is also elevated following mild neurotrauma, such as sport-related concussion.[16] 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.[17]

There has been a clarion call for more research focusing on TBI in older adults.[18] In Western countries, overall hospital admission rates for TBIs are decreasing, while admissions are *increasing* in patients over 65 years.[19-21] Falls are a common cause of ED visits[22, 23] and TBI[24-26] 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.[9, 27-39] 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 and 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).

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.[40] 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 because no neurophysiological assessments were undertaken. 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). None of the subjects had imaging evidence of moderate-severe brain atrophy. 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%. The trauma control sample was run at a different time than the MTBI sample, and the two runs were not calibrated for each other. Although the groups were analyzed in different runs, the control of the internal QC data showed that there were no differences between these runs and thus the results can be combined, and the approach will not unduly influence the results.

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). For exploratory purposes, a subgroup of patients aged 60 and older was compared to younger adults. 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 NF-L levels were not associated with the time between injury and blood sampling (r=-.072). 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). Again, for exploratory purposes, a subgroup of patients aged 60 and older was compared to younger adults. 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). Box and whisker plots comparing subgroups are illustrated in Figure 1.

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).

Figure 2 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, Figure 1, and Figure 2).

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.[12-14] It is recognized that TBI in older adults is understudied,[18] 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 a diagnostic or prognostic 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. *This study was not designed to be a diagnostic biomarker study*. The kinetics of NF-L are slower than other blood-based biomarkers, making it less useful during the first few hours following injury.[13, 16] This study was conceptualized to illustrate the associations between age, pre-injury neurological conditions, and acute mild head trauma on NF-L levels. 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 $\geq$ 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 on the day of injury.

Recent studies have illustrated an association between age and blood-based biomarkers.[41, 42] For example, S100B and GFAP levels have been shown to increase in relation to age. Calcagnile and colleagues concluded that older patients with acute MTBI (<65 versus ≥65 years) expressed significantly higher S100B levels.[42] Additionally, Gardner et al. reported that GFAP was less accurate for identifying CT-positive intracranial trauma among older versus younger MTBI patients.[41]

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,[9, 27-29] acute ischemic stroke,[30] active cerebral small vessel disease,[31] familial Alzheimer's disease,[32, 33] Huntington's disease,[34] frontotemporal dementia,[35] Creutzfeldt-Jakob disease,[36] Parkinsonian disorders,[37] other degenerative ataxias such as multiple system atrophy[38] and amyotrophic lateral sclerosis.[39]

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.[13, 16] 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 upon admission (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.

Acknowledgements: The authors acknowledge research assistant Anne Simi for her assistance with the patient enrolment and data collection at Tampere University Hospital, and research coordinator Annamari Aitolahti for her assistance with blood sample logistics. The authors thank TBIcare investigators Riikka S.K. Takala, Ari J. Katila, Janek Frantzén, Henna Ala-Seppälä, Anna Kyllönen, Henna-Riikka Maanpää and Jussi Tallus for their contribution in control patient recruitment at Turku University Hospital.

Author Disclosure Statement: Grant Iverson acknowledges unrestricted philanthropic support from the Mooney-Reed Charitable Foundation and ImPACT Applications, Inc. He serves as a strategic scientific advisor for BioDirection, Inc. Jussi Posti has received speaker's fees from Orion corporation and Finnish Medical Association. Dr. Blennow has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Merck, Novartis, Pfizer, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg.

Funding: The study was financially supported by the Finnish State Research Funding, and the Finnish Medical Society Duodecim. Dr. Luoto and Dr. Posti have received funding from Government's Special Financial Transfer tied to academic research in Health Sciences (Finland). Dr. Posti has received funding from the Emil Aaltonen Foundation sr and the Finnish Brain Foundation sr. Dr. Blennow acknowledges funding from The Torsten Söderberg Foundation, the Swedish Research Council, and the Swedish Brain Foundation. Dr. Zetterberg is a Wallenberg Academy Fellow and acknowledges support from the Swedish and European Research Council and the Dementia Research Institute at UCL.

References

1. Warner, M.A., Marquez de la Plata, C., Spence, J., Wang, J.Y., Harper, C., Moore, C., Devous, M. and Diaz-Arrastia, R. (2010). Assessing spatial relationships between axonal integrity, regional brain volumes, and neuropsychological outcomes after traumatic axonal injury. J. Neurotrauma 27, 2121-2130.
2. Ishikawa, H., Bischoff, R. and Holtzer, H. (1968). Mitosis and intermediate-sized filaments in developing skeletal muscle. J Cell Biol 38, 538-555.
3. Eyer, J. and Peterson, A. (1994). Neurofilament-deficient axons and perikaryal aggregates in viable transgenic mice expressing a neurofilament-beta-galactosidase fusion protein. Neuron 12, 389-405.
4. Lee, M.K., Xu, Z., Wong, P.C. and Cleveland, D.W. (1993). Neurofilaments are obligate heteropolymers in vivo. J Cell Biol 122, 1337-1350.
5. Heins, S., Wong, P.C., Muller, S., Goldie, K., Cleveland, D.W. and Aebi, U. (1993). The rod domain of NF-L determines neurofilament architecture, whereas the end domains specify filament assembly and network formation. J Cell Biol 123, 1517-1533.
6. Yates, D.M., Manser, C., De Vos, K.J., Shaw, C.E., McLoughlin, D.M. and Miller, C.C. (2009). Neurofilament subunit (NFL) head domain phosphorylation regulates axonal transport of neurofilaments. Eur J Cell Biol 88, 193-202.
7. Zetterberg, H., Smith, D.H. and Blennow, K. (2013). Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat. Rev. Neurol. 9, 201-210.
8. Schlaepfer, W.W., Lee, C., Trojanowski, J.Q. and Lee, V.M. (1984). Persistence of immunoreactive neurofilament protein breakdown products in transected rat sciatic nerve. J. Neurochem. 43, 857-864.

9. Disanto, G., Barro, C., Benkert, P., Naegelin, Y., Schadelin, S., Giardiello, A., Zecca, C., Blennow, K., Zetterberg, H., Leppert, D., Kappos, L., Gobbi, C., Kuhle, J. and Swiss Multiple Sclerosis Cohort Study, G. (2017). Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann. Neurol. 81, 857-870.

10. Brureau, A., Blanchard-Bregeon, V., Pech, C., Hamon, S., Chaillou, P., Guillemot, J.C., Barneoud, P., Bertrand, P., Pradier, L., Rooney, T. and Schussler, N. (2017). NF-L in cerebrospinal fluid and serum is a biomarker of neuronal damage in an inducible mouse model of neurodegeneration. Neurobiol. Dis. 104, 73-84.

11. Bacioglu, M., Maia, L.F., Preische, O., Schelle, J., Apel, A., Kaeser, S.A., Schweighauser, M., Eninger, T., Lambert, M., Pilotto, A., Shimshek, D.R., Neumann, U., Kahle, P.J., Staufenbiel, M., Neumann, M., Maetzler, W., Kuhle, J. and Jucker, M. (2016). Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases. Neuron 91, 56-66.

12. Al Nimer, F., Thelin, E., Nystrom, H., Dring, A.M., Svenningsson, A., Piehl, F., Nelson, D.W. and Bellander, B.M. (2015). Comparative Assessment of the Prognostic Value of Biomarkers in Traumatic Brain Injury Reveals an Independent Role for Serum Levels of Neurofilament Light. PLoS ONE 10, e0132177.

13. Shahim, P., Gren, M., Liman, V., Andreasson, U., Norgren, N., Tegner, Y., Mattsson, N., Andreasen, N., Ost, M., Zetterberg, H., Nellgard, B. and Blennow, K. (2016). Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. Sci Rep 6, 36791.

14. Korley, F.K., Yue, J.K., Wilson, D.H., Hrusovsky, K., Diaz-Arrastia, R., Ferguson, A.R., Yuh, E.L., Mukherjee, P., Wang, K.K.W., Valadka, A.B., Puccio, A.M., Okonkwo, D.O.

and Manley, G.T. (2018). Performance Evaluation of a Multiplex Assay for Simultaneous Detection of Four Clinically Relevant Traumatic Brain Injury Biomarkers. J. Neurotrauma.

15. Ljungqvist, J., Zetterberg, H., Mitsis, M., Blennow, K. and Skoglund, T. (2017). Serum Neurofilament Light Protein as a Marker for Diffuse Axonal Injury: Results from a Case Series Study. J. Neurotrauma 34, 1124-1127.
16. Shahim, P., Tegner, Y., Marklund, N., Blennow, K. and Zetterberg, H. (2018). Neurofilament light and tau as blood biomarkers for sports-related concussion. Neurology 90, e1780-e1788.
17. Oliver, J.M., Jones, M.T., Kirk, K.M., Gable, D.A., Repshas, J.T., Johnson, T.A., Andreasson, U., Norgren, N., Blennow, K. and Zetterberg, H. (2016). Serum Neurofilament Light in American Football Athletes over the Course of a Season. J. Neurotrauma 33, 1784-1789.
18. Gardner, R.C., Dams-O'Connor, K., Morrissey, M.R. and Manley, G.T. (2018). Geriatric Traumatic Brain Injury: Epidemiology, Outcomes, Knowledge Gaps, and Future Directions. J. Neurotrauma.
19. Koskinen, S. and Alaranta, H. (2008). Traumatic brain injury in Finland 1991-2005: a nationwide register study of hospitalized and fatal TBI. Brain Inj. 22, 205-214.
20. Perez, K., Novoa, A.M., Santamarina-Rubio, E., Narvaez, Y., Arrufat, V., Borrell, C., Cabeza, E., Cirera, E., Ferrando, J., Garcia-Altes, A., Gonzalez-Luque, J.C., Lizarbe, V., Martin-Cantera, C., Segui-Gomez, M., Suelves, J.M. and Working Group for Study of Injuries of Spanish Society of, E. (2012). Incidence trends of traumatic spinal cord injury and traumatic brain injury in Spain, 2000-2009. Accid. Anal. Prev. 46, 37-44.

21. Shivaji, T., Lee, A., Dougall, N., McMillan, T. and Stark, C. (2014). The epidemiology of hospital treated traumatic brain injury in Scotland. BMC Neurol. 14, 2.

22. Hoidrup, S., Sorensen, T.I., Gronbaek, M. and Schroll, M. (2003). Incidence and characteristics of falls leading to hospital treatment: a one-year population surveillance study of the Danish population aged 45 years and over. Scand J Public Health 31, 24-30.

23. Johansen, A., Dickens, J., Jones, M., Richmond, P. and Evans, R. (2011). Emergency department presentation following falls: development of a routine falls surveillance system. Emerg. Med. J. 28, 25-28.

24. Helling, T.S., Watkins, M., Evans, L.L., Nelson, P.W., Shook, J.W. and Van Way, C.W. (1999). Low falls: an underappreciated mechanism of injury. J. Trauma 46, 453-456.

25. Sarani, B., Temple-Lykens, B., Kim, P., Sonnad, S., Bergey, M., Pascual, J.L., Sims, C., Schwab, C.W. and Reilly, P. (2009). Factors associated with mortality and brain injury after falls from the standing position. J. Trauma 67, 954-958.

26. Poyry, T., Luoto, T.M., Kataja, A., Brander, A., Tenovuo, O., Iverson, G.L. and Ohman, J. (2013). Acute assessment of brain injuries in ground-level falls. J. Head Trauma Rehabil. 28, 89-97.

27. Kuhle, J., Barro, C., Disanto, G., Mathias, A., Soneson, C., Bonnier, G., Yaldizli, O., Regeniter, A., Derfuss, T., Canales, M., Schluep, M., Du Pasquier, R., Krueger, G. and Granziera, C. (2016). Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult. Scler. 22, 1550-1559.

28. Kuhle, J., Nourbakhsh, B., Grant, D., Morant, S., Barro, C., Yaldizli, O., Pelletier, D., Giovannoni, G., Waubant, E. and Gnanapavan, S. (2017). Serum neurofilament is

associated with progression of brain atrophy and disability in early MS. Neurology 88, 826-831.

29. Novakova, L., Zetterberg, H., Sundstrom, P., Axelsson, M., Khademi, M., Gunnarsson, M., Malmestrom, C., Svenningsson, A., Olsson, T., Piehl, F., Blennow, K. and Lycke, J. (2017). Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology 89, 2230-2237.

30. De Marchis, G.M., Katan, M., Barro, C., Fladt, J., Traenka, C., Seiffge, D.J., Hert, L., Gensicke, H., Disanto, G., Sutter, R., Peters, N., Sarikaya, H., Goeggel-Simonetti, B., El-Koussy, M., Engelter, S., Lyrer, P.A., Christ-Crain, M., Arnold, M., Kuhle, J. and Bonati, L.H. (2018). Serum neurofilament light chain in patients with acute cerebrovascular events. Eur. J. Neurol. 25, 562-568.

31. Gattringer, T., Pinter, D., Enzinger, C., Seifert-Held, T., Kneihsl, M., Fandler, S., Pichler, A., Barro, C., Grobke, S., Voortman, M., Pirpamer, L., Hofer, E., Ropele, S., Schmidt, R., Kuhle, J., Fazekas, F. and Khalil, M. (2017). Serum neurofilament light is sensitive to active cerebral small vessel disease. Neurology 89, 2108-2114.

32. Weston, P.S.J., Poole, T., Ryan, N.S., Nair, A., Liang, Y., Macpherson, K., Druyeh, R., Malone, I.B., Ahsan, R.L., Pemberton, H., Klimova, J., Mead, S., Blennow, K., Rossor, M.N., Schott, J.M., Zetterberg, H. and Fox, N.C. (2017). Serum neurofilament light in familial Alzheimer disease: A marker of early neurodegeneration. Neurology 89, 2167-2175.

33. Mattsson, N., Andreasson, U., Zetterberg, H., Blennow, K. and Alzheimer's Disease Neuroimaging, I. (2017). Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease. JAMA Neurol 74, 557-566.

34. Byrne, L.M., Rodrigues, F.B., Blennow, K., Durr, A., Leavitt, B.R., Roos, R.A.C., Scahill, R.I., Tabrizi, S.J., Zetterberg, H., Langbehn, D. and Wild, E.J. (2017). Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. Lancet Neurol. 16, 601-609.

35. Rohrer, J.D., Woollacott, I.O., Dick, K.M., Brotherhood, E., Gordon, E., Fellows, A., Toombs, J., Druyeh, R., Cardoso, M.J., Ourselin, S., Nicholas, J.M., Norgren, N., Mead, S., Andreasson, U., Blennow, K., Schott, J.M., Fox, N.C., Warren, J.D. and Zetterberg, H. (2016). Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology 87, 1329-1336.

36. Thompson, A.G.B., Luk, C., Heslegrave, A.J., Zetterberg, H., Mead, S.H., Collinge, J. and Jackson, G.S. (2018). Neurofilament light chain and tau concentrations are markedly increased in the serum of patients with sporadic Creutzfeldt-Jakob disease, and tau correlates with rate of disease progression. J. Neurol. Neurosurg. Psychiatry.

37. Hansson, O., Janelidze, S., Hall, S., Magdalinou, N., Lees, A.J., Andreasson, U., Norgren, N., Linder, J., Forsgren, L., Constantinescu, R., Zetterberg, H., Blennow, K. and Swedish Bio, F.s. (2017). Blood-based NfL: A biomarker for differential diagnosis of parkinsonian disorder. Neurology 88, 930-937.

38. Wilke, C., Bender, F., Hayer, S.N., Brockmann, K., Schols, L., Kuhle, J. and Synofzik, M. (2018). Serum neurofilament light is increased in multiple system atrophy of cerebellar type and in repeat-expansion spinocerebellar ataxias: a pilot study. J. Neurol.

39. Feneberg, E., Oeckl, P., Steinacker, P., Verde, F., Barro, C., Van Damme, P., Gray, E., Grosskreutz, J., Jardel, C., Kuhle, J., Koerner, S., Lamari, F., Amador, M.D.M., Mayer, B., Morelli, C., Muckova, P., Petri, S., Poesen, K., Raaphorst, J., Salachas, F., Silani, V.,

Stubendorff, B., Turner, M.R., Verbeek, M.M., Weishaupt, J.H., Weydt, P., Ludolph, A.C. and Otto, M. (2018). Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis. Neurology 90, e22-e30.

40. Posti, J.P., Hossain, I., Takala, R.S., Liedes, H., Newcombe, V., Outtrim, J., Katila, A.J., Frantzen, J., Ala-Seppala, H., Coles, J.P., Kyllonen, A., Maanpaa, H.R., Tallus, J., Hutchinson, P.J., van Gils, M., Menon, D.K. and Tenovuo, O. (2017). Glial Fibrillary Acidic Protein and Ubiquitin C-Terminal Hydrolase-L1 Are Not Specific Biomarkers for Mild CT-Negative Traumatic Brain Injury. J. Neurotrauma.

41. Gardner, R.C., Rubenstein, R., Wang, K.K.W., Korley, F.K., Yue, J.K., Yuh, E.L., Mukherje, P., Valadka, A.B., Okonkwo, D.O., Diaz-Arrastia, R. and Manley, G.T. (2018). Age-Related Differences in Diagnostic Accuracy of Plasma Glial Fibrillary Acidic Protein and Tau for Identifying Acute Intracranial Trauma on Computed Tomography: A TRACK-TBI Study. J. Neurotrauma 35, 2341-2350.

42. Calcagnile, O., Holmen, A., Chew, M. and Unden, J. (2013). S100B levels are affected by older age but not by alcohol intoxication following mild traumatic brain injury. Scand J Trauma Resusc Emerg Med 21, 52.

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)

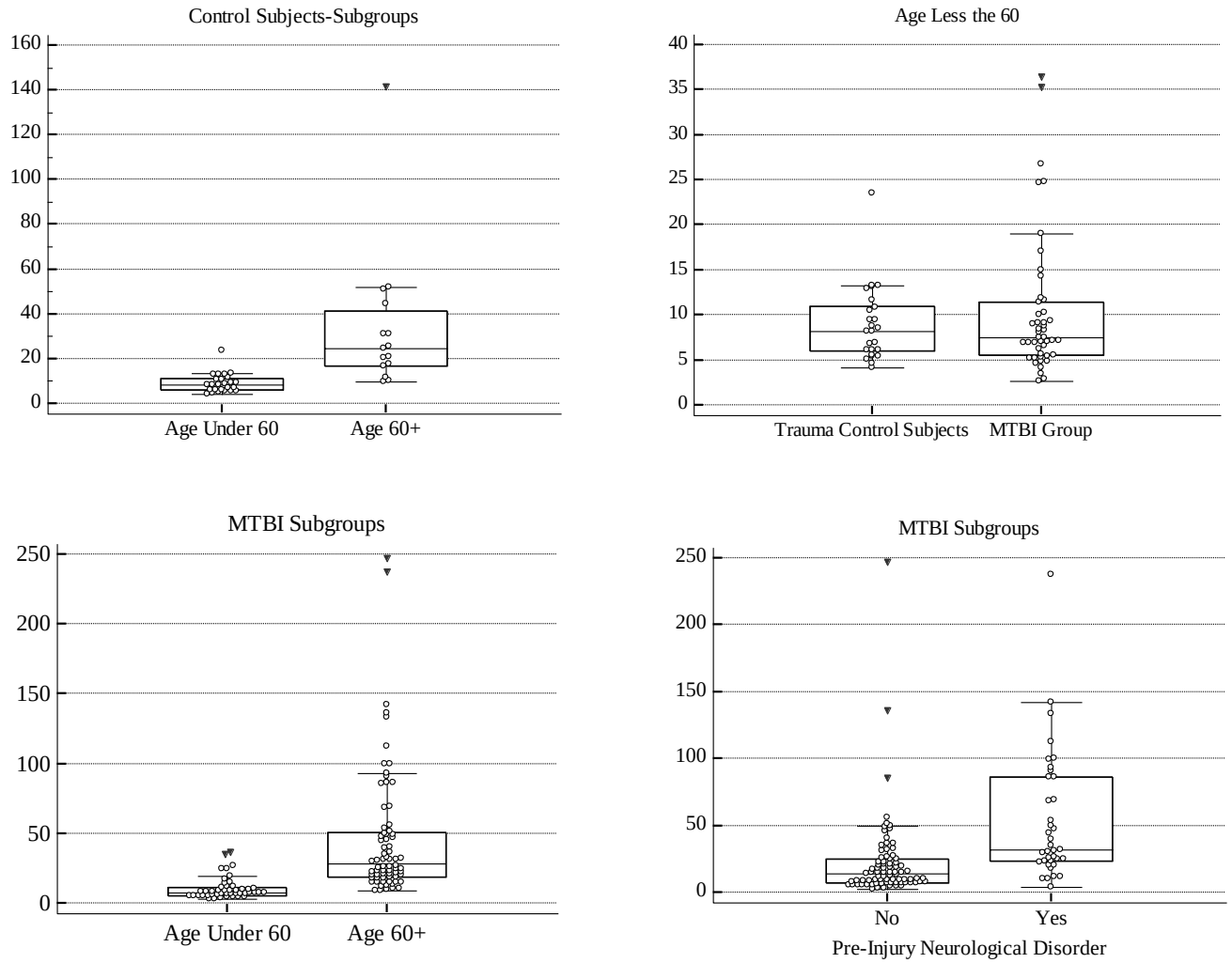
Note: N=118. Some subjects have more than one diagnosis.

Table 2. Descriptive statistics for NF-L (pg/mL) 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

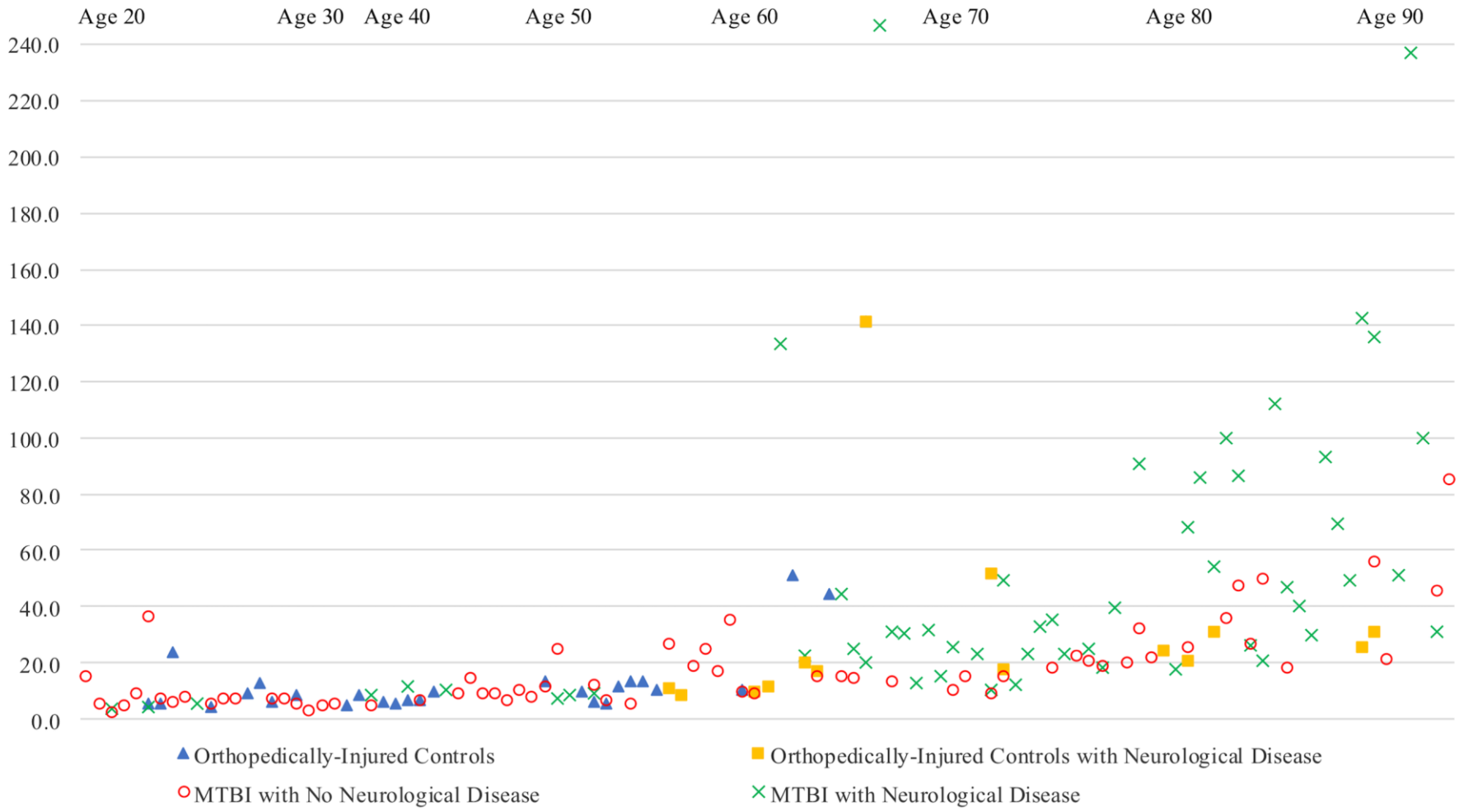
Note: *The average ages for control subjects were as follows: No Neurological Conditions=M=36.2, Md=33.5, SD=11.5; Neurological Conditions Unknown=M=57.1, Md=55.0, SD=15.4; and Pre-Injury Neurological Conditions=M=66.2, Md=65.0, SD=13.9.

Figure 1. Box and whisker plots comparing subgroups.



Note: Subgroup sample sizes are presented in Table 2. The range on the y-axis varies across graphs in association with outliers.

Figure 2. Neurofilament light levels in adults and older adults sorted by age.



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.