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Letter to the Editor

Association of admission serum levels of neurofilament light chain and in-hospital mortality in geriatric patients with COVID-19

Francesca Marchegiani[1], Rina Recchioni[1], Fiorella Marcheselli[1], Mirko Di Rosa[2], Jacopo Sabbatinelli[3,4], Giulia Matacchione[3], Angelica Giuliani[3], Deborah Ramini[1], Pierpaolo Stripoli[1], Leonardo Biscetti[5], Giuseppe Pelliccioni[5], Riccardo Sarzani[3,6], Francesco Spannella[3,6], Antonio Cherubini[7], Andrea Corsonello[2,8], Antonio Domenico Procopio[1,3], Anna Rita Bonfigli[9], Massimiliano Bonafè[10], Fabrizia Lattanzio[9] and Fabiola Olivieri[1,3]

[1] Clinic of Laboratory and Precision Medicine, IRCCS INRCA, Ancona, Italy

[2] Unit of Geriatric Pharmacoepidemiology and Biostatistics, IRCCS INRCA, Ancona, Italy

[3] Department of Clinical and Molecular Sciences (DISCLIMO), Università Politecnica Delle Marche, Ancona, Italy

[4] Laboratory Medicine Unit, Azienda Ospedaliero Universitaria "Ospedali Riuniti", Ancona, Italy

[5] Neurology Department, IRCCS INRCA, Ancona, Italy

[6] Internal Medicine and Geriatrics, IRCCS INRCA, Ancona, Italy

[7] Geriatria, Accettazione Geriatrica e Centro Di Ricerca Per L'invecchiamento, IRCCS INRCA, Ancona, Italy

[8] Unit of Geriatric Medicine, IRCCS INRCA, Cosenza, Italy

[9] Scientific Direction, IRCCS INRCA, Ancona, Italy

[10] Department of Experimental, Diagnostic, and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy

Corresponding authors:

Jacopo Sabbatinelli, MD, PhD (j.sabbatinelli@staff.univpm.it)

Giulia Matacchione, PhD (g.matacchione@staff.univpm.it)

Department of Clinical and Molecular Sciences

Università Politecnica delle Marche

Via Tronto 10/A – 60126 Ancona, Italy

Phone: +39.071.2206144

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The severity of coronavirus disease 19 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection, can range from very mild to severe symptoms, depending on the multi-organ damage. Even though direct SARS-CoV-2 infection of the brain parenchyma remains a matter of debate, a wide range of neurological manifestations, including delirium, anosmia, encephalopathy, encephalitis, and cerebrovascular manifestations was reported in COVID-19 patients **(Meppiel et al., 2021; Pezzini and Padovani, 2020)**. Recent data raised the possibility that patients affected by COVID-19-associated neurological syndromes exhibit impaired amyloid processing linked to neuronal injury and neuroinflammation **(Ziff et al., 2022)**. Inflammatory biomarkers were frequently detected at high concentrations in cerebrospinal fluid (CSF) of patients with COVID-19 neurological syndromes, suggesting that neuronal damage occurs, probably with long-term consequences that are still unknown **(Domingues et al., 2022)**. Among the CSF biomarkers associated with neuronal injury, the neurofilament light chain (NfL) is attracting the attention of researchers and clinicians. NfL is an essential cytoskeleton protein of the axons, not specific to the central nervous system (CNS) impairment but also associated with peripheral neuropathy **(Hayashi et al., 2021)**. With a low molecular weight of 68 kDa, NfL can easily diffuse to CSF and blood. The recent development of ultrasensitive assays, such as single molecule array (SIMOA) and Next Generation ELISA, allows reproducible measurement of NfL concentrations in serum or plasma **(O'Connell et al., 2019)**. It was demonstrated that NfL in both CSF and serum reflects the temporal pattern of the post-ischemic axonal injury **(Pujol-Calderon et al., 2019)**. Determining the extent of neuronal injury in COVID-19 patients is essential to better understand disease pathophysiology and to evaluate potential therapies. In adult patients affected by COVID-19, increased circulating levels of NfL were associated with acute disease severity **(Ameres et al., 2020; Prudencio et al., 2021)**, also in the absence of major neurological manifestations **(Verde et al., 2022)**. However, several questions remain unanswered, especially in the oldest patients affected by multiple comorbidities, including neurodegenerative diseases and renal dysfunction, that could affect NfL circulating levels **(Polymeris et al., 2022; Rebelos et al., 2022)**.

Here, we assessed admission levels of serum NfL (sNfL) in geriatric patients with COVID-19 with the aim to evaluate the ability of circulating NfL to improve COVID-19 mortality prediction alone and/or in association with other demographic, biochemical, and molecular circulating biomarkers.

The present study was performed on a subset of samples from the already published Report-Age COVID observational study, which was conducted at the Italian National Center on Aging (IRCCS INRCA, Ancona, Italy) with the aim of understanding the predictors of adverse outcomes in older patients hospitalized and diagnosed with COVID-19 **(Olivieri et al., 2022)**. Samples were collected between 1st March 2020 and 24th June 2021. The study was conducted in adherence to the Declaration of Helsinki and has been approved by the Ethics Committee of the IRCCS INRCA (reference number CE-INRCA-20008; ClinicalTrials.gov, NCT04348396). All the included patients had been confirmed to be infected with SARS-CoV-2 by real-time reverse transcriptase-polymerase chain reaction assay regardless of the clinical symptoms. Informed consent was obtained from all individual participants included in the study. Demographic and anamnestic data, biochemical and hematological variables, information on treatments, comorbidities, and survival were collected in a retrospective manner and anonymized before release, as previously described **(Olivieri et al., 2022)**. The Clinical Frailty Scale (CFS), an ordinal 9-point scale in which the assessor evaluates the degree of frailty from clinical data, was employed to assess frailty **(Rockwood and Theou, 2020)**.

sNfL levels were determined using commercially available kits for the Simple Plex™ Ella microfluidic platform (Protein Simple, Bio-Techne, Minneapolis, USA). Samples were prepared following the manufacturer's instructions. Briefly, samples were diluted by mixing 25 µL of the sample with 25 µL of the Sample Diluent provided. All samples were run in triplicate and the mean value was considered for the analysis. Levels of NfL were measured within the linear range of each assay. Samples with an intra-assay coefficient of variation below 10.0% were included in this study. The lower limit of quantification is 2.7 pg/mL, and the upper limit is 10290 pg/mL.

Continuous variables were reported as either mean and standard deviation or median and interquartile range based on their normal or non-normal distribution (assessed using the Shapiro-Wilk test), respectively. The clinical and laboratory characteristics of the study cohort upon admission were compared according to categorized sNfL using the Chi-squared test. Optimal sNfL cutoffs for predicting survival were computed using the Evaluate Cutpoints R package. Comparison of variables between groups was performed by one-way analysis of variance or Kruskal-Wallis equality-of-populations rank test as appropriate. The association between sNfL and mortality was initially investigated by Kaplan-Meier curves and statistical significance was assessed by the log-rank test for equality of survivor functions. Crude and adjusted Cox proportional hazards

models were built to derive hazard ratios (HR) and 95% confidence intervals (95% CI) of the association between all independent variables and the study outcome. The length of hospital stay was used as the time to failure variable for the model. Sequential imputation using chained equations with linear regression method was applied in case of missing values in covariates. The imputation model included age, gender, and comorbidities. A two-tailed P-value <0.05 was considered significant. Data were analyzed using STATA version15.1 Statistical Software Package for Windows (Stata Corp, College Station, TX).

A total of 205 consecutive geriatric patients, median age (IQR) 86 (82-91) years, who were hospitalized at the INRCA hospital (Ancona, Italy) due to COVID-19 were included in the analysis. 30.2% (62/205) of the enrolled patients deceased during their in-hospital stay. The mean time from hospital admission to discharge was 20.1±11.4 days for survivor patients and 14.6±12.3 days for deceased patients. The minimum number of days for which patients in the recovered group remained hospitalized was one day, while the maximum hospital stay was 53 days for survived patients and 56 days for deceased patients.

With respect to medication, most of the patients received corticosteroids during their hospital stay (n=142; 69.3%); with no significant difference (p=0.986) between deceased (n=43; 69.4%) and survived (n=99; 69.2%). First, two cutoffs, i.e., 85 and 258 pg/mL, were computed to maximize the ability of sNfL to predict survival in our cohort. Patients were then categorized in three groups with sNfL <85 pg/mL (low levels), 85 – 258 pg/mL (intermediate levels), and >258 pg/mL (high levels). The clinical and laboratory characteristics of the study cohort at admission according to the NfL categories are reported in **Table 1**.

The proportion of deceased COVID-19 patients was higher among patients with high levels of sNfL (50%), and this group of patients was characterized by higher median age, prevalence of dementia and chronic kidney diseases (CKD), higher levels of D-dimer, NT-proBNP, procalcitonin, interleukin-6 (IL-6), and lower estimated glomerular filtration rate (CKD-EPI eGFR) and potassium **(Table 1)**. Spearman's correlations with Bonferroni correction revealed that NfL serum levels were positively related to age and NT-proBNP and negatively associated with eGFR **(Table 2)**.

The Kaplan-Meier survival estimates and log-rank tests for comparison for COVID-19 patients grouped based on serum NfL, reported in **Figure 1**, showed that patients with admission sNfL higher than 85 pg/ml (intermediate and high levels) had increased mortality compared to those with sNfL lower than 85 pg/ml.

Finally, adjusted Cox regressions models evaluating the predictive value of sNfL, age, and gender (model 1) and model 1 plus CFS (model 2), showed that old age, high sNfL levels and high CFS (≥8) were the best independent predictors of in-hospital mortality in geriatric patients affected by COVID-19 **(Table 3).** When model 2 was adjusted also for the routine laboratory parameters that differed significantly among the three groups of patients (**Table 1**) i.e., eGFR, D-dimer, potassium, NT-proBNP, procalcitonin and IL-6 (model 3), old age and low eGFR, but not sNfL, were independent predictors of in-hospital mortality (**Table 3**).

Based on previous findings that established NfL as a marker of axonal injury **(Khalil et al., 2018)**, we investigated whether sNfL measured in hospitalized geriatric patients affected by COVID-19 could provide clinical guidance to assess the risk of in-hospital mortality. We observed that a significantly increased number of deceased COVID-19 geriatric patients showed high levels of sNfL, and this group of patients was characterized by the oldest age, the highest prevalence of dementia, CKD and frailty, the highest levels of D-dimer, NT-proBNP, procalcitonin, IL-6 and the lowest levels of potassium. However, in the fully adjusted Cox regression model, only age and eGFR showed a significant association with an increased risk of in-hospital mortality. Our results are in line with recent data suggesting that renal function should be regarded as a potential confounder when assessing circulating NfL levels, since impaired filtration can affect NfL concentration **(Polymeris et al., 2022; Rebelos et al., 2022)**. Previous reports showed that circulating NfL levels are negatively related to eGFR in healthy subjects and patients with type 2 diabetes, suggesting that the effect of age on circulating NfL could be partially mediated by eGFR **(Akamine et al., 2020; Ladang et al., 2022)**. On the other hand, Hermansson et al. reported that plasma NfL did not correlate with serum creatinine among patients infected with HIV with a mean age of 40 years, suggesting that the relationship between blood NfL and renal function becomes more evident among older adults **(Hermansson et al., 2019)**. As previously demonstrated in Parkinson's Disease **(Niemann et al., 2021)**, sNfL showed a direct correlation with NT-proBNP. While the assessment of this cardiac biomarker in patients with neurodegenerative disorders proved useful in the assessment of subclinical cardiac damage, this close interrelationship confirms that NfL accumulates with advancing age and declining renal function, in a way similar to NT-proBNP. Notably, it has been previously shown that sNfL enhances the predictive value of NT-proBNP as a biomarker of long-term cardiovascular outcome after ischemic stroke **(Uphaus et al., 2019)** and that sNfL was associated with worse

cognitive performance in patients with heart failure **(Polymeris et al., 2020)**. To this regard, the direct correlation between sNfL and NT-proBNP observed in our cohort could reflect a post-ischemic neuronal damage due to impaired cerebral perfusion in patients with reduced cardiac output and renal function. Additionally, in previous investigations the association between sNfL and eGFR was driven by the subjects with obesity whereas no such correlation was found in lean individuals **(Akamine et al., 2020; Polymeris et al., 2022)**. Unfortunately, no data were available regarding the BMI of geriatric COVID-19 patients enrolled in our study, and we were unable to test this association.

Notably, sNfL levels were not correlated with the most common routinary analyzed biomarkers that we identified as associated with increased short-term mortality risk in this setting of patients in our previous report, i.e. neutrophil, lymphocyte, and eosinophil count, blood glucose, C-reactive protein, procalcitonin, and ferritin **(Olivieri et al., 2022)**.

Our study has some limitations that need to be addressed, most notably its retrospective nature, single-center design, and limited sample size. Moreover, no CSF specimens were available for NfL analysis, which could have provided a more accurate assessment of the cerebral milieu. Indeed, it is still much debated whether SARS-CoV-2 can invade the CNS. Nevertheless, we have found a strong correlation between the highest sNfL levels and the presence of dementia. This is in line with our previous report addressing the association between cerebrospinal fluid NfL levels and neurodegenerative disorders in the geriatric population **(Marchegiani et al., 2019)**.

In conclusion, serum NfL could be associated with severe outcomes in geriatric patients affected by COVID-19, even if caution should be used when interpreting these findings in patients with impaired renal function. Future studies are warranted to confirm its independent prognostic value in hospitalized geriatric patients.

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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TABLES

Table 1. Sample description.

	Total	**sNfL<85 pg/mL**	**sNfL: 85-258 pg/mL**	**sNfL>258 pg/mL**	**p**
	N=205	**N=58**	**N=101**	**N=46**	
Female gender, n (%)	122 (59.5%)	34 (58.6%)	57 (56.4%)	31 (67.4%)	0.449
Age (years)	**86 (82-91)**	**83 (80-87)**	**87 (83-91)**	**87.5 (84-93)**	**<0.001**
Length of stay (days)	16 (9-26)	17 (10-27)	16 (11-25)	13.5 (7-25)	0.422
Dead, n (%)	**62 (30.2%)**	**7 (12.1%)**	**32 (31.7%)**	**23 (50%)**	**<0.001**
CFS, n (%)					**<0.001**
1-3	40 (19.5%)	22 (37.9%)	15 (14.9%)	3 (6.5%)	
4-7	101 (49.3%)	26 (44.8%)	52 (51.5%)	23 (50%)	
8-9	64 (31.2%)	10 (17.2%)	34 (33.7%)	20 (43.5%)	
Comorbidities					
History of myocardial infarction, n (%)	23 (11.2%)	8 (13.8%)	12 (11.9%)	3 (6.5%)	0.485
Dementia, n (%)	**91 (44.4%)**	**13 (22.4%)**	**45 (44.6%)**	**33 (71.7%)**	**<0.001**
CKD, n (%)	**64 (31.2%)**	**5 (8.6%)**	**38 (37.6%)**	**21 (45.7%)**	**<0.001**
Hypertension, n (%)	154 (75.1%)	45 (77.6%)	71 (70.3%)	38 (82.6%)	0.243
Stroke, n (%)	23 (11.2%)	4 (6.9%)	15 (14.9%)	4 (8.7%)	0.257
COPD, n (%)	38 (18.5%)	8 (13.8%)	19 (18.8%)	11 (23.9%)	0.417
Atrial Fibrillation, n (%)	56 (27.3%)	17 (29.3%)	24 (23.8%)	15 (32.6%)	0.495
Cancer, n (%)	41 (20%)	11 (19%)	22 (21.8%)	8 (17.4%)	0.805
CHF, n (%)	53 (25.9%)	11 (19%)	28 (27.7%)	14 (30.4%)	0.346
Diabetes, n (%)	50 (24.4%)	13 (22.4%)	26 (25.7%)	11 (23.9%)	0.892
Laboratory parameters					
Hemoglobin (g/dL)	11.4 (10.0-12.6)	11.9 (10.4-13.1)	11.3 (10.0-12.4)	10.9 (9.6-12.5)	0.083
Neutrophil %	76.5 (65.5-85.8)	74.1 (66.7-83.6)	76.3 (64.9-85.9)	79.0 (66.4-87.7)	0.535
Lymphocyte %	15.6 (9.3-22.7)	15.6 (9.9-24.2)	16.4 (9.3-23.4)	13.9 (8.1-20.1)	0.396
Eosinophil %	0.2 (0.0-1.0)	0.2 (0.0-1.2)	0.3 (0.0-1.0)	0.3 (0.0-1.0)	0.857
Basophil %	0.1 (0.1-0.2)	0.2 (0.1-0.3)	0.1 (0.1-0.2)	0.1 (0.0-0.2)	0.275
Neutrophils ($\times 10^3$/ µL)	5.5 (3.9-8.0)	5.1 (4.0-7.5)	5.5 (3.8-8.0)	6.3 (4.3-10.8)	0.183
Lymphocytes ($\times 10^3$/ µL)	1.2 (0.8-1.6)	1.1 (0.8-1.6)	1.2 (0.8-1.7)	1.2 (0.8-1.6)	0.930
Eosinophils ($\times 10^3$/ µL)	0.03 (0.00-0.09)	0.03 (0.00-0.10)	0.01 (0.00-0.10)	0.03 (0.00-0.09)	0.648
Basophils ($\times 10^3$/ µL)	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.990
D-dimer (ng/mL)	**1260 (750-2320)**	**930 (675-1620)**	**1305 (740-2510)**	**1720 (980-3360)**	**0.006**
eGFR (mL/min)	**64.5 (45.0-83.0)**	**80.0 (59.0-89.0)**	**60.0 (40.0-79.0)**	**47.5 (30.5-81.0)**	**<0.001**
Fasting glucose (mg/dL)	105.0 (82.0-132.0)	114.0 (98.0-130.0)	103.0 (80.0-138.0)	98.0 (71.0-128.0)	0.221
Sodium (mmol/L)	139.0 (136.0-143.0)	139.0 (137.0-142.0)	139.0 (136.0-143.0)	140.0 (135.5-147.5)	0.491
Potassium (mmol/L)	**4.1 (3.6-4.6)**	**4.2 (3.9-4.7)**	**4.2 (3.7-4.6)**	**3.9 (3.4-4.3)**	**0.013**
NT-proBNP (pg/mL)	**1551 (670-4174)**	**830 (340-2085)**	**1598.5 (696.5-3948.5)**	**2305 (1400-7777)**	**0.001**
CRP (mg/dL)	2.9 (1.2-8.1)	1.9 (0.6-7.4)	3.2 (1.5-8.1)	3.7 (1.6-11.1)	0.097
Procalcitonin (ng/mL)	**0.080 (0.050-0.220)**	**0.050 (0.050-0.145)**	**0.080 (0.050-0.175)**	**0.145 (0.070-0.860)**	**<0.001**
IL-6 (pg/mL)	**34.6 (12.6-73.1)**	**25.8 (8.2-65.8)**	**32.6 (13.3-69.5)**	**61.3 (29.7-166.0)**	**0.011**

Ferritin (ng/mL)	548.0 (310.0-902.0)	452.0 (278.0-805.0)	583.0 (310.0-907.0)	574.5 (427.0-982.0)	0.284
NLR	4.9 (3.0-8.9)	4.3 (2.7-7.8)	4.4 (2.9-9.3)	5.7 (3.5-11.0)	0.242
dNLR	1.9 (0.8-4.0)	1.3 (0.4-3.2)	2.0 (0.9-4.0)	2.1 (0.6-4.3)	0.249
PLR	202.3 (134.1-301.3)	228.9 (146.4-301.3)	196.0 (130.9-298.9)	242.7 (115.3-304.3)	0.580
LMR	2.4 (1.6-3.6)	2.3 (1.6-3.5)	2.4 (1.6-3.4)	2.5 (1.7-4.3)	0.390

Data are median (IQR) for continuous variables or number (%) for categorical variables. p-values for Chi-squared tests. In bold significant associations. CFS, clinical frailty scale; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; NLR, neutrophil-to-lymphocyte ratio; dNLR, derived NLR; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; sNfL, serum neurofilament light chain.

Table 2. Spearman's rank correlation coefficients with Bonferroni correction for multiple comparisons.

	sNfL	**CFS**
sNfL	1	
CFS	0.4529*	1
Age	0.3995*	0.4148*
Hemoglobin	-0.1890	0.0003
Neutrophil %	0.1227	0.1315
Lymphocyte %	-0.1773	-0.2001
Eosinophil %	-0.0257	-0.0443
Basophil %	-0.1395	-0.0434
Neutrophils ($\times 10^3$ /μL)	0.1776	0.2301
Lymphocytes ($\times 10^3$/μL)	-0.0761	-0.0234
Eosinophils ($\times 10^3$/μL)	0.0274	-0.1134
Basophils ($\times 10^3$ /μL)	0.0123	-0.0538
NLR	0.1662	0.2097
dNLR	-0.0288	0.1309
PLR	-0.0247	0.0058
LMR	-0.0064	0.0951
D-dimer	0.2451	0.1215
eGFR	-0.4117*	-0.2509
Fasting glucose	-0.1935	-0.0467
Serum sodium	0.1039	0.1781
Serum potassium	-0.1677	-0.0421
NT-proBNP	0.3266*	0.2364
C-reactive protein	0.2178	0.2351
Procalcitonin	0.2927	0.1544
IL-6	0.2648	0.1306
Ferritin	0.1655	0.1160

* $p<0.005$. In bold significant associations. CFS, clinical frailty scale; NLR, neutrophil-to-lymphocyte ratio; dNLR, derived NLR; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

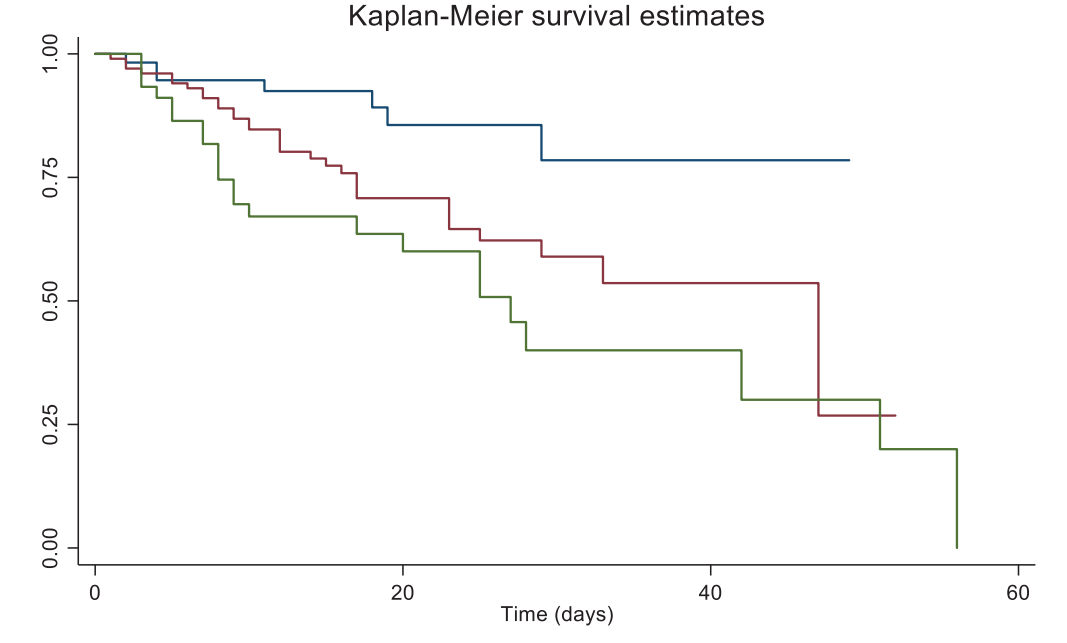
Table 3. Cox proportional hazards models (n=205).

	Model 1	**Model 2**	**Model 3**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Serum NfL (pg/mL), ref. <85			
85-258	1.99 (0.87-4.55)	1.76 (0.76-4.06)	1.35 (0.56-3.23)
>258	**3.38 (1.42-8.04)**	**2.88 (1.20-6.90)**	1.92 (0.73-5.05)
Female gender	0.86 (0.51-1.47)	0.75 (0.43-1.28)	0.83 (0.47-1.48)
Age (years)	**1.11 (1.05-1.16)**	**1.09 (1.03-1.15)**	**1.10 (1.04-1.16)**
CFS, ref. 1-3			
4-7		2.49 (0.94-6.61)	2.31 (0.83-6.44)
8-9		**3.17 (1.15-8.76)**	2.40 (0.79-7.25)
D-dimer (ng/mL)			1.00 (0.99-1.00)
eGFR (mL/min)			**0.98 (0.97-0.99)**
Potassium (mmol/L)			1.04 (0.67-1.61)
NT-proBNP (mg/L)			1.00 (0.99-1.00)
Procalcitonin (ng/mL)			1.05 (0.99-1.11)
IL-6 (pg/mL)			1.00 (0.99-1.00)

CFS, clinical frailty scale; CKD, chronic kidney disease; CHF, congestive heart failure; eGFR, estimated glomerular filtration rate; NfL, neurofilament light chain.

FIGURE LEGEND

Fig. 1 Kaplan-Meier survival estimates for categorized serum NfL


Kaplan-Meier survival estimates
1.00
0.75
0.50
0.25
0.00
0
20
40
60
Time (days)
Serum NfL <85 pg/mL
Serum NfL 85 - 258 pg/mL
Serum NfL >258 pg/mL
Log-rank test for equality of survivor functions:
χ² = 12.54; p = 0.002