Prognostic performance of blood neurofilament light chain protein in hospitalised adults with COVID-19: an individual participant data meta-analysis

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ABSTRACT

Background and aims: To investigate the prognostic value of blood neurofilament light chain protein (NfL) in the acute phase of hospitalised patients with COVID-19.

Methods: We conducted an individual participant data (IPD) meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline. Articles have been screened on MEDLINE (PubMed) and Scopus from database inception to May 23rd 2022. We included studies containing IPD from hospitalised adult COVID-19 patients with a measurement of blood NfL in the acute phase and data regarding at least one clinical outcome among ICU admission, need of mechanical ventilation (MV) and death. We derived the age-adjusted measures NfL Z-scores and conducted mixed-effects modelling to test associations between NfL Z-scores and other variables, including clinical outcomes. Summary receiver operating characteristic curves (SROCs) were used to calculate the area under the curve (AUC) for blood NfL.

Results: We identified 382 records, of which 7 studies were included with 707 COVID-19 cases (mean age 66.2 ± 14.7 years, 68.4% males) who met the inclusion criteria. Median NfL Z-score (2.41, IQR: 1.22 - 3.10) was elevated compared to age-matched reference population. NfL Z-scores were significantly associated with most clinical and laboratory parameters, including disease duration and severity. Higher NfL Z-scores were associated with higher rates of ICU admission, MV and death, even after adjustment for covariates. SROCs revealed AUCs of 0.73, 0.80 and 0.82 for mortality, MV and ICU admission respectively.

Conclusions: Blood NfL levels were elevated in individuals with COVID-19 and were associated with clinical severity parameters. However, the limited prognostic value of the marker prevents its use alone as a routine biomarker in hospitalised COVID-19 patients.

Key messages

What is already known on this topic

Neurofilament light chain protein (NfL) is a blood biomarker of neuroaxonal damage, the levels of which are increased in hospitalised COVID-19 patients and seem to be associated with poor clinical outcome.

What this study adds

In this individual participant meta-analysis, we investigated the prognostic value of blood NfL in a large multicentric cohort of 707 COVID-19 patients and found strong associations between marker levels and clinical outcome (ICU admission, need of mechanical ventilation and death), although with a mostly fair prognostic accuracy.

How this study might affect research, practice or policy

Given its limited prognostic value, future studies should evaluate the combination of blood NfL with other potential surrogate parameters of COVID-19 severity in prognostic scores useful for clinical practice.

INTRODUCTION

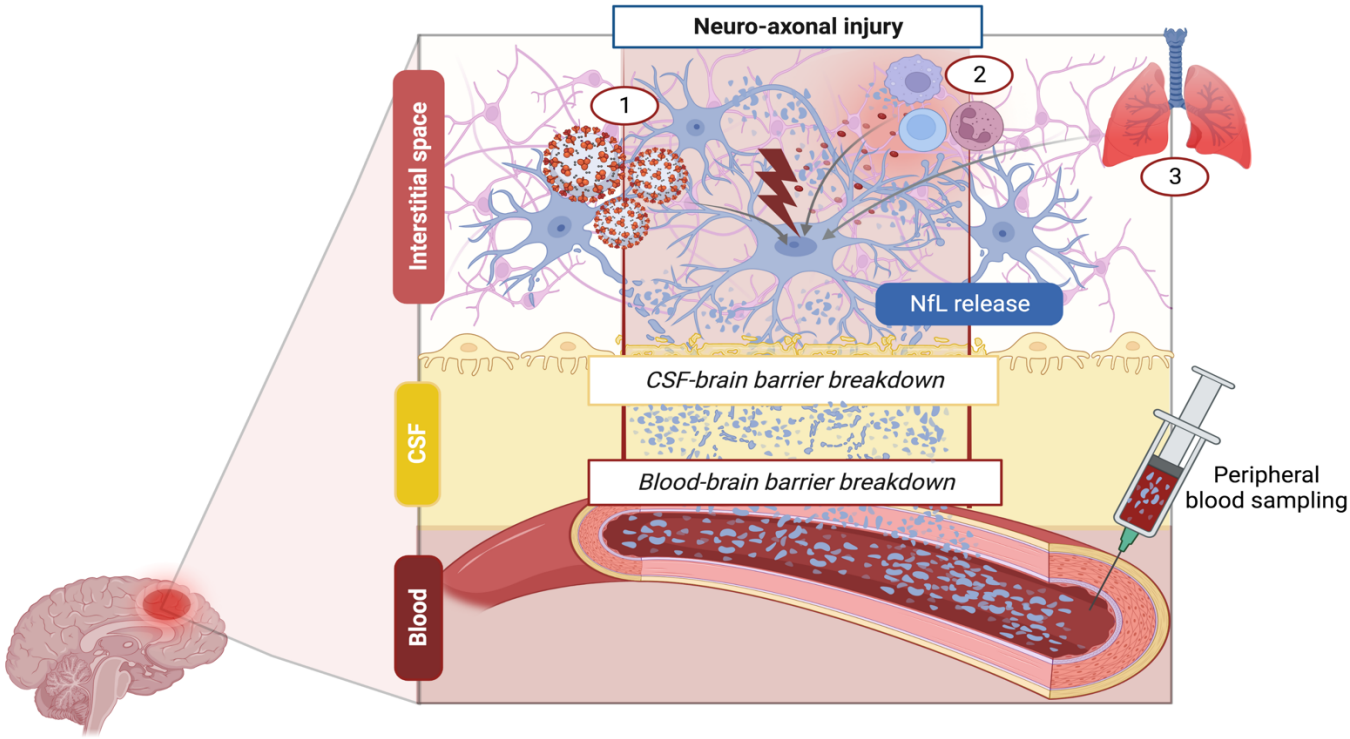
Since the outbreak of the pandemic in January 2020, the management of COVID-19 has rapidly become a priority in all healthcare organisations worldwide (**Pezzini NatRev 2020; Ellul Lancet 2020; Mao JAMA 2020, Guan NEJM 2020**). COVID-19 is a systemic disease primary affecting the respiratory system, although 30% of all patients complain about central and peripheral neurological manifestations (**Pezzini, NatRev 2020; Ellul, Lancet 2020; Mao JAMA 2020, Guan NEJM 2020**). In this regard, neuronal damage may occur as a consequence of various pathogenetic pathways, such as direct viral invasion, cytokines storm, para- or post-infectious autoimmunity and secondary effects of a severe multi-organ dysfunction (**Li JMedVirol 2020, Baig Chem Neurosci 2020**). Recently, the introduction of new ultrasensitive immunoassays has allowed the assessment of blood neuronal and glial biomarkers, as correlates of CNS involvement, in large and longitudinal cohorts of primary and non-primary neurological diseases, including COVID-19 (**Khalil 2018, Abu Rumeileh 2022, Abdelhak 2022**). In particular, neurofilament light chain protein (NfL) has gained significant attention as a biochemical correlate of neuroaxonal involvement, given its ability to accurately track subclinical axonal pathology, monitor disease course and predict long-term outcomes in different neurological and systemic conditions (**Khalil 2018, Abu Rumeileh 2022,**).

The preliminary evidence of increased NfL levels in COVID-19 cases with only mild-to-moderate (e.g., anosmia, headache) or without specific neurological symptoms (**Ameres M, JOON 2020, Mariotto JNNP 2020**) suggested that a subtle neuronal damage might be even more frequent and still underestimated in COVID-19. On the other side, patients with severe COVID-19 showed a sustained NfL elevation at follow up, possibly reflecting a persistent CNS involvement (**Kanberg N Neurol 2020, Kanberg 2021, Cooper 2020**) (**Figure 1).** Most interestingly, both prospective and cross-sectional studies have demonstrated an association between NfL and unfavourable clinical outcomes, encompassing death, intensive care unit (ICU) admission, and mechanical ventilation in hospitalized COVID-19 patients (**Sutter R Ann Neurol 2020, Kanberg N Neurol 2020, 2021, Mariotto JNNP 2020, De Lorenzo JON 2021, Aamodt JON 2021**).

However, NfL median levels varied largely among different studies, probably as a consequence of heterogeneous inclusion criteria across studies and a lack of systematic NfL value adjustment according to

confounding factors such as age and renal function (**Benkert et al., Lancet Neurology, 2022, Joshua 2022, Harp 2022, Fitzgerald 2022, Rubsamen 2022, Akamine 2020, Abu-Rumeileh 2022, Hay 2021**). Considering all these issues, we conducted an individual participant data (IPD) meta-analysis to test whether the measurement of blood NfL levels in the acute phase of COVID-19 may aid prognostication in hospitalised cases with COVID-19.

Figure 1. Mechanisms leading to blood neurofilament light chain (NfL) increase in COVID-19. Neuro-axonal injury in COVID-19 may result from the interplay between different pathophysiological mechanisms including 1) potentially direct viral invasion, 2) pro-inflammatory cytokine release and autoimmunity, 3) secondary damage due to systemic impairment (e.g., hypoxia for concomitant COVID-19-related pneumonia). NfL are first released in the central nervous system (CNS) interstitium, then they are drained in the cerebrospinal fluid (CSF) and finally reach the bloodstream. In COVID-19 a sustained blood NfL increase could be enhanced also by concomitant blood-brain barrier breakdown due to inflammatory and hypoxia-related mechanisms. **(Abu-Rumeileh Brain 2022)**



METHODS

IPD meta-analysis protocol followed the Preferred Reporting Items for Systematic Reviews and meta-analysis guidelines for IPD systematic reviews (PRISMA-IPD) (**Stewart et al. 2015 ref**) and was registered with PROSPERO (CRD42022358924).

Search strategy and selection criteria

Six authors (MF, AA, SAR, LB, RO, MR) systematically searched MEDLINE (PubMed) and Scopus for articles published from databases inception to May 23rd 2022 addressing the role of biomarkers in predicting the outcomes of interest. An a priori search string was developed with 3-steps Delphi method to include terms for (i) NfL as a biomarker and (ii) COVID-19 as disease of interest (**Supplementary Material for full search string).** Results were restricted to original articles in English, German or Italian language. A priori criteria for inclusion were: 1) hospitalised patients 2) age ≥18 years, 3) COVID-19 diagnosis (PCR or radiology-based), 4) a measurement of blood NfL during the acute phase and 5) available data regarding at least one outcome among ICU admission, need of mechanical ventilation and mortality (primary outcome). Only COVID-19 cases without distinct neurological syndromes were included, therefore excluding all cases of encephalitis, stroke or other major neurological complications related to COVID-19. We included only studies with sufficient information to calculate the age- adjusted NfL Z-score (see below). Study selection was conducted on Rayyan platform (rayyan.ai). Titles and abstracts were screened independently. Potentially relevant articles were acquired in full text and assessed for eligibility by the same six authors working in pairs. The final selection was shared among all the six authors. Disagreements were resolved by consensus.

Data extraction and processing

We invited authors of the included studies to participate by providing IPD on a standardized collection tool (**supplementary materials, under preparation**). IPD comprised both data reported in the included studies as well as unpublished data meeting our inclusion criteria (collected during the study enrolment but then excluded from the authors). Contributors ensured local ethical, regulatory and data sharing agreement were in place. We collected demographic, comorbidities and COVID-19 severity defined according to the World Health Organization (WHO) criteria for the clinical management of COVID-19 (**Who website**); timing (i.e., days onset to admission and to blood collection), biological matrix (plasma or serum) (**Supplementary table 1**) and values of NfL, PaO2/FiO2 ratio and other laboratory parameters (absolute lymphocyte and neutrophil count, lactate dehydrogenase – LDH and C-reactive protein [CRP] – PCR, creatinine levels). Submitted datasets were processed by two investigators (FC, MF) to harmonise data recording across studies in accordance with pre-defined variable types. If a contributor was unable to harmonise data with our format, we allowed to report the original study data; these data were extracted and fully checked by two reviewers (FC, MF) with a standardised approached, then the harmonisation was shared with all investigators.

Bias assessment

Quality assessment was performed with the Newcastle-Ottawa scale (NOS) (**Luchini 2017**). NOS includes assessment of selection of cohort explored, control cohort, length and adequacy of observation, as well as comparability of control and experimental cohorts. We summarized the assessment as low, moderate, or high according to the overall score achieved by each study.

Statistical Analysis

Statistical analyses were carried out with IBM SPSS Statistics V.21 (IBM, Armonk USA), GraphPad Prism V.7 (GraphPad Software, La Jolla, California, USA) and R version 4.2.2 (R-project, Vienna, Austria).

As NfL correlates with age (**Benkert et al., Lancet Neurology, 2022, Abu-Rumeileh 2022**), NfL age-adjusted Z-score were calculated using available large reference database (n=4532 samples from control persons) (**Benkert et al., Lancet Neurology, 2022**). Generalized Additive Model for Location, Scale and Shape (GAMLSS model) was used to model NfL variations with age and to derive individual NfL Z-score, a continuous measure indicating how strongly (in terms of number of standards deviations) the adjusted NfL value deviates from levels in healthy controls. NfL plasma values were converted into corresponding serum values for Z score calculations according to a published equation (**Benkert lancet 2022**).

Meta-analysis was conducted using mixed-effects modelling, with center/study implemented as random effect. Generalized linear mixed-effects models (GLMMs) were applied to test associations between NfL Z-scores, clinical features, laboratory values, potential prognostic variables (sex, PaO2/FiO2, hypertension, diabetes, neutrophil and lymphocyte absolute count, creatinine, LDH, CRP) (**Izcovich A et al., other papers)** and binary clinical outcomes (ICU admission, need for mechanical ventilation, death). Those variables which tested significative at univariate analysis were added to multivariate GLMMs. For each model, we reported data on estimate coefficient and/or odd ratio (OR) as well as p-values.

The lack of a common cut-off among studies precluded the traditional bivariate models for accuracy testing. First, we investigate the performance of NfL in the assessment of poor outcome by means of receiver-operating characteristic analysis by calculating the area under (AUC) the receiver-operating curves (ROCs) derived from GLMMs. Second, we used a 2-stage random-effects model integrating multiple thresholds within each study to calculate a summary ROC (SROC) from meta-analysis of diagnostic accuracy. For both methods, we estimated optimal thresholds through maximized Youden index (sensitivity+specificity-1) or through Youden Index after weighting specificity at 75%, 85%, and 95%, defined a-priori as progressive reasonable threshold for prognostications, and we reported each respective sensitivity. All analyses were considered statistically significant with $p<0.05$.

RESULTS

We identified 382 records by database searches. Seven studies reached final stage, providing IPD for 893 hospitalised COVID-19 cases. A total of 707 patients referred to 7 centers (Oslo n=26, Drammen n=20, Milan n=104, Uppsala n=19, Brescia n=370, Basel n=26, Jacksonville n=142) met the inclusion criteria and were included in the analysis **(Supplementary figures 1 and 2).**

Cohort description

Demographical, clinical and laboratory features of included cohorts are reported in **Table 1 and supplementary table 2**. Mean age at blood sampling was 66.2±14.7 years (males=470, 68.4%), mean disease duration from symptom onset to admission was 6.8±5.1 day, and from onset to biomarker assessment was and 9.6±10.6 days Hypertension (26.5%) and diabetes (15.2%) were the most common cardiovascular risk factors in the whole cohort. Included patients were mainly diagnosed with critical disease course (424/469 with available classification, 91.4%), while 13 (2.8%) and 27 (5.8%) had moderate and severe disease, respectively. Elevated CRP and LDH values, a normal neutrophil count and low absolute lymphocyte count were typical findings in COVID-19 patients (**Table 1**). Median NfL Z-scores were higher than 0 in the included cohorts (median: 2.41; interquartile range, IQR: 1.22 - 3.10), ranging from a min of -3.72 to a max of 4.17 (**Supplementary Figure 3**). The median hospitalization time was 15 days (IQR: 6 - 32). During the hospital stay, 74 out of 223 patients (33.2%) required MV with a median ventilation duration of 7.5 days (IQR: 3 - 16). Moreover, 323 out of 657 patients (49.9%) were admitted at the ICU. Finally, data on survival were available for all patients and death occurred in 198 cases (28.0%). Death was associated to COVID-19 itself and its related complications.

Table 1. Demographical, clinical and laboratory features of analysed COVID-19 patients.

	Whole cohort (n=707)	
	Value	**n. cases (%)**
Sex (female/male) (frequency (%)]	217 (31.6) / 470 (68.4)	687 (97.2)
Age [mean (± sd) (range)]	66.2 (± 14.7) (22.0 - 99.1)	707 (100.0)
NfL (pg/ml) [median (IQR) (range)]	37.8 (17.1 - 78.4) (2.3 - 2233.2)	707 (100.0)
NfL Z-scores [median (IQR) (range)]	2.41 (1.22 - 3.10) (-3.72 - 4.17)	707 (100.0)
Current disease		
Time to admission (days) [median (IQR)]	7 (4 - 9)	264 (37.3)
Time to blood sampling (days) [median (IQR)]	7 (2 - 14)	422 (59.7)
COVID-19 severity (moderate/severe/critical) [frequency (%)]	13 (2.8) / 27 (5.8) / 429 (91.4)	469 (66.3)
Medical history		
Diabetes (yes/no) [frequency (%)]	62 (15.2) / 347 (84.8)	409 (57.9)
Hypertension (yes/no) [frequency (%)]	113 (26.5) / 313 (73.5)	426 (60.3)
Laboratory analyses		

Lymphocyte count (x10^9/l) [median (IQR)]	0.90 (0.56 - 1.30)	285 (40.3)
Neutrophil count (x10^9/l) [median (IQR)]	5.2 (3.5 - 8.0)	152 (21.5)
CRP (mg/l) [median (IQR)]	68.2 (16.6 - 137.8)	338 (47.8)
LDH (U/l) [median (IQR)]	334 (257 - 452)	247 (34.9)
Creatinine (mg/dl) [median (IQR)]	0.83 (0.72 -1.04)	149 (21.1)
PaO2/FiO2 [median (IQR)]	225 (106 - 326)	141 (19.9)
Clinical outcomes		
Days of hospitalization [median (IQR)]	15 (6 - 32)	506 (71.6)
Mechanical ventilation (yes/no) [frequency (%)]	74 (31.8) / 159 (68.2)	233 (33.0)
Days of mechanical ventilation [median (IQR)]	7.5 (3 - 16)	52 (7.4)
ICU admission (yes/no) [frequency (%)]	323 (49.9) / 324 (51.1)	647 (91.5)
Death (yes/no) [frequency (%)]	198 (28.0) / 509 (72.0)	707 (100.0)

Associations between NfL, clinical and laboratory variables

We found significant associations between NfL Z-scores and most clinical and laboratory variables (**Table 2**). In particular, NfL Z-scores were significantly associated with disease duration (time from onset to blood marker assessment) ($p<0.0001$) as well as with COVID-severity ($p=0.0001$, Table 2). Moreover, history of hypertension ($p=0.020$) or diabetes mellitus ($p<0.0001$) was significantly related to higher NfL biomarker values. Among laboratory parameters, CRP ($p=0.009$], LDH ($p=0.005$), creatinine ($p<0.001$) but not absolute lymphocyte and neutrophil counts were related to NfL Z-scores. Low values of PaO2/FiO2 ratio associated with higher NfL Z-scores ($p=0.0003$).

Table 2. Associations between NfL Z-scores and other demographical, laboratory and clinical variables (univariate GLMM, center as random effect).

Univariate GLMM analysis	Estimate (95%CI)	OR (95%CI)	p-value	n. cases
Male Sex	0.157 (-0.051 - 0.366)	1.171 (0.950 - 1.442)	0.139	687
Current disease				
Time to admission	-0.007 (-0.037 - 0.020)	0.993 (0.967 - 1.02)	0.621	264
Time to blood sampling	0.040 (0.025 - 0.055)	1.041 (1.025 - 1.057)	<0.0001	422
COVID-19 severity	0.692 (0.366 - 1.017)	1.997 (1.442 - 2.764)	0.0001	469
Medical history				
Diabetes	0.678 (0.369 - 0.986)	1.969 (1.447 - 2.680)	<0.0001	409
Hypertension	0.302 (0.048 - 0.556)	1.353 (1.049 - 1.744)	0.02	426

Laboratory analyses				
Lymphocytes	0.019 (-0.055 - 0.094)	1.019 (0.946 - 1.098)	0.645	285
Neutrophils	0.005 (-0.022 - 0.031)	1.005 (0.977 - 1.031)	0.736	152
CRP	0.0018 (0.0005 - 0.0032)	1.002 (1.0005 - 1.003)	0.009	338
LDH	0.0009 (0.0003 - 0.0015)	1.0009 (1.0003 - 1.002)	0.0052	247
Creatinine	0.948 (0.613 - 1.283)	2.580 (1.845 - 3.609)	<0.0001	149
PaO2FiO2	-0.0028 (-0.0042 - -0.0013)	0.997 (0.996 - 0.999)	0.0003	142

Associations between NfL and clinical outcome measures

In the whole cohort, univariate GLMM analyses identified NfL-Z score, sex, COVID-19 duration (days from symptoms onset), severity at blood collection, and history of diabetes, as variables associated with ICU admission in COVID-19 cases (**Table 3**). In the multivariate GLMM, NfL Z-score remained a significant independent predictor of ICU admission even after accounting for covariates **(Table 3**). When the need of MV was considered as outcome, NfL-Z score, sex, disease duration at blood collection, and CRP resulted significant predictors in the univariate GLMM (**Table 4**). Similarly, in the multivariate GLMM, higher NfL Z-score values were significantly associated with MV (**Table 4**). Further, NfL-Z score, days from onset to admission, COVID-19 severity, diabetes, lymphocyte count, CRP, LDH, creatinine and PaO2/FiO2 ratio were significantly associated with death as outcome at univariate GLMM analysis (**Table 5**). After accounting for covariates, NfL Z-score was confirmed as a significant negative prognostic factor for survival (**Table 5**).

Table 3. Associations between NfL, other variables and ICU admission (recruiting center as random effect).

Univariate GLMM analysis	**Estimate difference (95%CI)**	**OR (95%CI)**	**z-value**	**p-value**	**n. cases**
Male Sex	0.754 (0.384 - 1.124)	2.13 (1.47 - 3.08)	3.9934	0.0001	627
NfL Z-scores	0.383 (0.245 - 0.521)	1.47 (1.28 - 1.68)	5.4458	<0.0001	647
Current disease					
Time to admission	0.021 (-0.036 - 0.079)	1.02 (0.96 - 1.08)	0.727	0.47	227
Time to blood sampling	0.094 (0.057 - 0.132)	1.10 (1.06 - 1.14)	4.8827	<0.0001	385
COVID-19 severity	3.673 (3.671 - 3.676)	39.38 (39.28 - 39.48)	2796.7	<0.0001	409
Medical history					
Diabetes	1.044 (0.432 - 1.656)	2.84 (1.54 - 5.24)	3.3452	0.0008	409
Hypertension	-0.021 (-0.487 - 0.445)	0.98 (0.61 - 1.56)	-0.0893	0.93	426
Laboratory analyses					

Lymphocytes	-0.036 (-0.252 - 0.180)	0.96 (0.78 - 1.20)	-0.3248	0.75	251
Neutrophils	0.020 (-0.033 - 0.073)	1.02 (0.97 - 1.08)	0.751	0.45	152
CRP	0.003 (-0.0003 - 0.006)	1.003 (0.9997 - 1.006)	1.7527	0.0797	303
LDH	0.004 (0.002 - 0.006)	1.004 (1.002 - 1.006)	4.0676	<0.0001	213
Creatinine	0.666 (-0.053 - 1.384)	1.95 (0.95 - 3.99)	1.8163	0.069	149
PaO2FiO2	-0.011 (-0.016 - -0.006)	0.99 (0.98 - 0.994)	-4.5994	<0.0001	142
Multivariate GLMM analysis	**Estimate difference (95%CI)**	**OR (95%CI)**	**z-value**	**p-value**	**n. cases**
Model 1					
NfL Z-scores	0.374 (0.232 - 0.515)	1.45 (1.26 - 1.67)	5.172	<0.0001	627
Male sex	0.711 (0.332 - 1.091)	2.04 (1.39 - 2.98)	3.6708	0.0002	
Model 2					
NfL Z-scores	0.504 (0.306 - 0.702)	1.66 (1.36 - 2.02)	4.9872	<0.0001	381
Male sex	0.580 (0.082 - 1.079)	1.79 (1.09 - 2.94)	2.2821	0.023	
Time to blood sampling	0.067 (0.031 - 0.102)	1.07 (1.03 - 1.11)	3.6859	0.0002	
Model 3					
NfL Z-scores	1.011 (0.279 - 1.743)	2.75 (1.32 - 5.71)	2.7061	0.0068	93
Male sex	0.396 (-1.069 - 1.861)	1.49 (0.34 - 6.43)	0.5302	0.596	
Time to blood sampling	0.088 (0.027 - 0.149)	1.09 (1.03 - 1.16)	2.8334	0.0046	
COVID-19 severity	0.789 (-0.689 - 2.266)	2.20 (0.50 - 9.64)	1.0458	0.296	
Diabetes	2.109 (-0.072 - 4.29)	8.24 (0.93 - 72.96)	1.8955	0.058	

Table 4. Associations of NfL Z-scores and other variables with the need of mechanical ventilation (recruiting center as random effect).

Univariate GLMM analysis	**Estimate difference (95%CI)**	**OR (95%CI)**	**z-value**	**p-value**	**n. cases**
Male Sex	1.006 (0.244 - 1.768)	2.74 (1.28 - 5.86)	2.5886	0.0096	213
NfL Z-scores	0.786 (0.481 - 1.090)	2.19 (1.62 - 2.98)	5.052	<0.0001	233
Current disease					
Time to admission	-0.108 (-0.409 - 0.194)	0.90 (0.66 - 1.21)	-0.6988	0.48	30
Time to blood sampling	0.085 (0.033 - 0.136)	1.09 (1.03 - 1.15)	3.2334	0.0012	191
COVID-19 severity	1.110 (-0.495 - 2.715)	3.03 (0.61 - 15.10)	1.3552	0.18	99
Medical history					
Diabetes	-	-	-	-	only one center
Hypertension	-0.635 (-3.091 - 1.822)	0.53 (0.05 - 6.19)	-0.5062	0.61	36
Laboratory analyses					
Lymphocytes	0.048 (-0.136 - 0.232)	1.05 (0.87 - 1.26)	0.5072	0.61	41
Neutrophils	0.212 (-0.068 - 0.491)	1.24 (0.93 - 1.63)	1.4867	0.14	59

CRP	0.016 (0.007 - 0.0257)	1.02 (1.01 - 1.03)	3.3129	0.0009	87
LDH	-	-	-	-	no data
Creatinine	1.136 (-0.166 - 2.439)	3.12 (0.85 - 11.46)	1.7101	0.087	45
PaO2FiO2	0.025 (-0.014 - 0.065)	1.03 (0.99 - 1.07)	1.2565	0.21	41
Multivariate analysis	**Estimate difference (95%CI)**	**OR (95%CI)**	**z-value**	**p-value**	**n. cases**
Model 1					
NfL Z-scores	0.845 (0.507 - 1.182)	2.33 (1.66 - 3.26)	4.9088	<0.0001	213
Male sex	0.958 (0.121 - 1.794)	2.61 (1.13 - 6.02)	2.2441	0.025	
Model 2					
NfL Z-scores	0.968 (0.582 - 1.354)	2.63 (1.79 - 3.87)	4.9159	<0.0001	187
Male sex	0.937 (-0.012 - 1.887)	2.55 (0.99 - 6.60)	1.935	0.053	
Time to blood sampling	0.050 (0.004 - 0.096)	1.05 (1.004 - 1.10)	2.1181	0.034	

Table 5. Associations between NfL, other variables and death (recruiting center as random effect).

Univariate GLMM analysis	**Estimate difference (95%CI)**	**OR (95%CI)**	**z-value**	**p-value**	**n. cases**
Male Sex	0.243 (-0.149 - 0.635)	1.28 (0.86 - 1.89)	1.2162	0.22	687
NfL Z-scores	0.688 (0.4972 - 0.8785)	1.99 (1.64 - 2.41)	7.0722	<0.0001	707
Current disease					
Time to admission	-0.113 (-0.186 - -0.040)	0.89 (0.83 - 0.96)	-3.0473	0.0023	264
Time to blood sampling	-0.002 (-0.030 - 0.027)	0.998 (0.97 - 1.03)	-0.1112	0.91	422
COVID-19 severity	1.280 (0.283 - 2.277)	3.60 (1.33 - 9.74)	2.5163	0.012	469
Medical history					
Diabetes	1.285 (0.690 - 1.881)	3.62 (1.99 - 6.56)	4.2302	<0.0001	409
Hypertension	0.367 (-0.124 - 0.858)	1.44 (0.88 - 2.36)	1.467	0.14	426
Laboratory analyses					
Lymphocytes	-0.608 (-1.060 - -0.156)	0.54 (0.35 - 0.86)	-2.6383	0.008	285
Neutrophils	0.005 (-0.055 - 0.065)	1.005 (0.95 - 1.07)	0.1567	0.88	152
CRP	0.005 (0.002 - 0.008)	1.005 (1.002 - 1.008)	3.3395	0.0008	338
LDH	0.004 (0.002 - 0.006)	1.004 (1.002 - 1.006)	4.0661	<0.0001	247
Creatinine	1.192 (0.372 - 2.011)	3.30 (1.45 - 7.47)	2.8508	0.0044	149
PaO2FiO2	-0.004 (-0.008 - -0.001)	0.996 (0.992 - 0.999)	-2.2537	0.024	142
Multivariate GLMM analysis	**Estimate difference (95%CI)**	**OR (95%CI)**	**z-value**	**p-value**	**n. cases**
Model 1					
NfL Z-scores	0.588 (0.373 - 0.803)	1.80 (1.45 - 2.23)	5.363	<0.0001	469
COVID-19 severity	1.059 (-0.113 - 2.230)	2.88 (0.89 - 9.30)	1.7715	0.077	
Model 2					
NfL Z-scores	1.134 (0.326 - 1.942)	3.11 (1.39 - 6.97)	2.7521	0.0059	108
COVID-19 severity	8.501 (-426.88 - 443.881)	>100 (0 - >1000)	0.0383	0.97	
Lymphocytes	-1.792 (-2.981 - -0.603)	0.17 (0.05 - 0.55)	-2.9548	0.0031	
Diabetes	1.783 (0.623 - 2.942)	5.94 (1.86 - 18.96)	3.013	0.0026	

ROC curve analyses for NfL Z-score

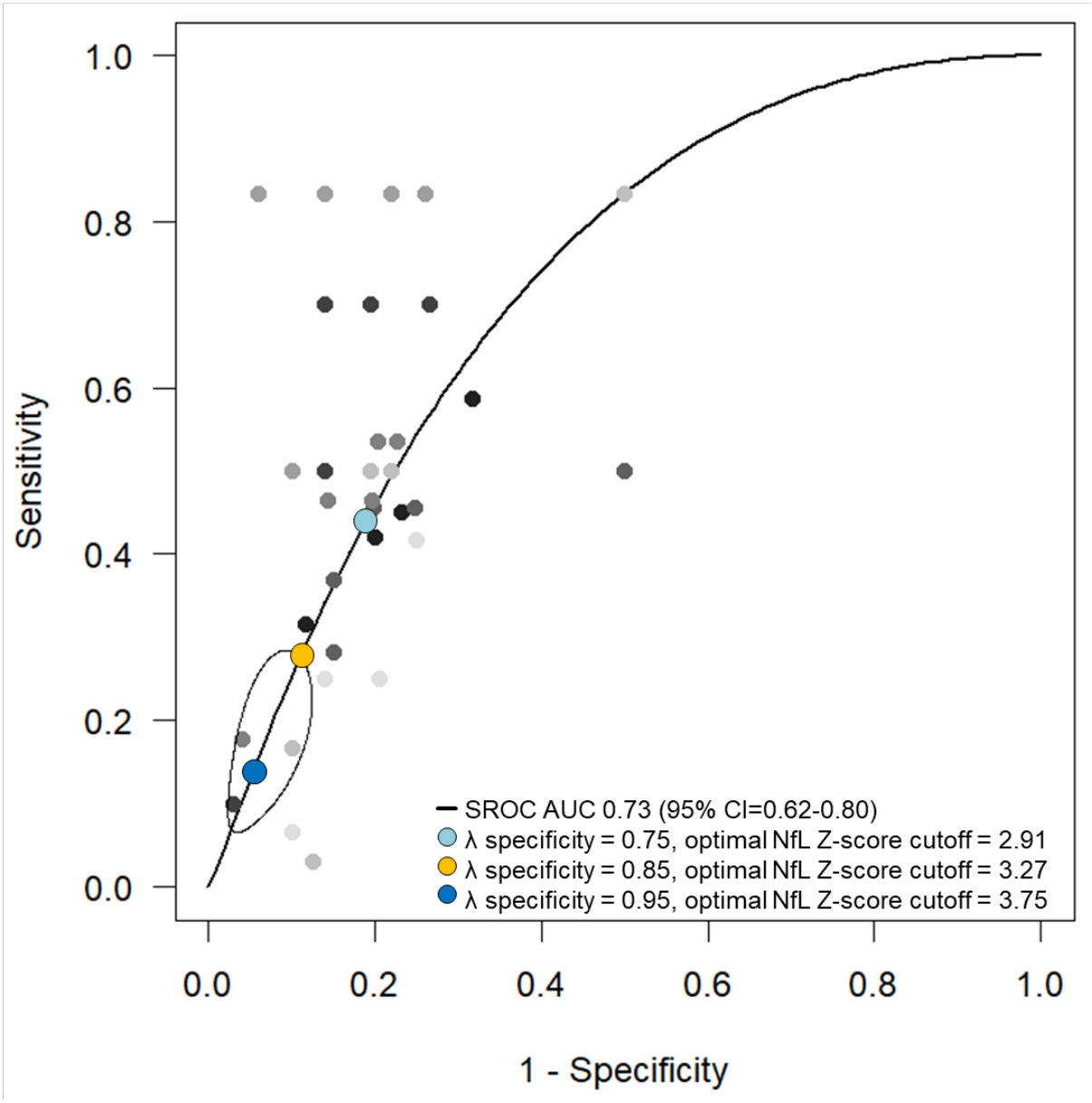
In the ROC analyses derived from univariate and multivariate GLMMs, the performance of NfL Z-score to discriminate patients with poor outcome from those with good outcome was moderate yielding an AUC >0.70 (**Table 6**). The best accuracy was yielded by the multivariate GLMM, which included NfL-Z score, sex, time to blood sampling, COVID-19 severity and diabetes as variables in the prediction of ICU admission (AUC 0.916) (**Table 6**). SROCs for NfL Z-score as a biomarker and mortality as primary outcome showed an AUC of 0.73 (95% CI= 0.62-0.80, **Figure 2**). After setting specificity at 75%, 85% and 95%, the optimal NfL Z-score threshold was 2.91 (sensitivity 0.44. specificity 0.81), 3.28 (sensitivity 0.29, specificity 0.88), and 3.75 (sensitivity 0.15, specificity 0.95; **Figure 2**), respectively. At maximized Youden Index a cut-off of 2.08 yielded sensitivity of 77% and a specificity of 58%. SROCs for mechanical ventilation (AUC 0.80, 95% CI=0.64-0.89) and ICU admission (AUC 0.82, 95% CI=0.72-0.90) showed also a fair predictive value of NfL Z-score, with sensitivity being low at a-priori set specificity boundaries (**supplementary Figure 4**).

Table 6. Receiver operating characteristic analyses for models with NfL-Z scores.

Outcome	Model	Variables	AUC (95%CI)	75% Specificity			85% Specificity			95% Specificity			max Youden's index		
				Cut-off	Spec	Sens	Cut-off	Spec	Sens	Cut-off	Spec	Sens	Cut-off	Spec	Sens
ICU admission	Univariate model	NfL Z-score	0.728 (0.690-0.767)	0.584	0.753	0.536	0.617	0.852	0.430	0.667	0.951	0.279	0.539	0.710	0.647
	Multivatiate model 1	NfL Z-score + sex	0.740 (0.702-0.778)	0.554	0.751	0.569	0.643	0.851	0.421	0.697	0.951	0.270	0.535	0.725	0.623
	Multivatiate model 2	NfL Z-score + sex + time to blood sampling	0.786 (0.740-0.833)	0.482	0.754	0.667	0.534	0.850	0.580	0.650	0.952	0.460	0.545	0.874	0.580
	Multivariate model 3	NfL Z-score + sex + time to blood sampling + COVID-19 severity + diabetes	0.916 (0.861-0.971)	0.608	0.750	0.892	0.740	0.857	0.800	0.792	0.964	0.738	0.792	0.964	0.738
Mechanical ventilation	Univariate model	NfL Z-score	0.877 (0.827-0.927)	0.282	0.767	0.838	0.367	0.855	0.757	0.540	0.956	0.568	0.420	0.912	0.730
	Multivatiate model 1	NfL Z-score + sex	0.896 (0.849-0.942)	0.250	0.755	0.886	0.355	0.860	0.814	0.612	0.951	0.571	0.294	0.804	0.871
	Multivatiate model 2	NfL Z-score + sex + time to blood sampling	0.881 (0.820-0.942)	0.218	0.755	0.813	0.305	0.856	0.792	0.522	0.957	0.604	0.350	0.892	0.792
Death	Univariate model	NfL Z-score	0.767 (0.730-0.803)	0.370	0.752	0.611	0.442	0.851	0.455	0.545	0.957	0.232	0.282	0.656	0.783
	Multivatiate model 1	NfL Z-score + COVID-19 severity	0.715 (0.668 - 0.763)	0.446	0.756	0.553	0.494	0.856	0.371	0.554	0.96	0.200	0.430	0.702	0.629

	Multivatiate model 2	NfL Z-score + COVID-19 severity + lymphocytes + diabetes	0.882 (0.822 - 0.942)	0.430	0.75	0.75	0.580	0.859	0.659	0.700	0.953	0.614	0.223	0.594	0.977

Figure 2. Receiver operating characteristic curves for the diagnostic accuracy of NfL Z-score for predicting mortality.



DISCUSSION

This is the first IPD meta-analysis investigating the prognostic role of blood NfL in a large and comprehensive cohort of 707 hospitalised adult patients with COVID-19, admitted to 7 hospitals worldwide.

We showed that blood NfL values were typically elevated in hospitalised COVID-19 patients compared to large age-adjusted datasets of healthy controls (**Benkert et al., Lancet Neurology, 2022**). Similar to previous reports, NfL Z-scores correlated significantly with clinical severity as higher values were found in most severe cases (**Needham Brain 2022, Kanberg 2021**). The significant associations between NfL Z-scores and CRP or PaO2/FiO2 ratio, an established measure of lung injury severity, may support the complex interplay between hypoxic injury, inflammatory response and other mechanisms that contribute to neuronal loss in COVID-19 (**Kanberg 2021 and 2020, Smeele 2022**). Taking together, all these data suggest that the rise of the biomarker in blood might reflect the degree of a multifactorial neuroaxonal damage occurring in the acute phase, which, in turn, relates to disease severity.

Most interestingly, we provided here evidence about the strong associations between higher blood NfL values and higher rates of MV, ICU admission and death in hospitalised COVID-19 cases. Of note, NfL remained a good independent predictor of unfavourable outcome, even after adjustment for covariates. Our results are in line with recent findings in patients admitted to ICU after cardiac arrest or due to sepsis-associated encephalopathy, in whom blood NfL values were significantly associated with disease severity and clinical outcome (**Paper Jama AC, Abu Rumeileh 2022, Ehler 2019**). Nevertheless, a very recent and large cohort study from two centres (**Smeele Brain comm 2022**) found contradicting results in regard to the association between blood NfL and mortality in hospitalised COVID-19 patients. Nevertheless, Smeele et al. postulated a potential relationship between faster mortality and NfL values assessed at admission but not during disease. In line with this hypothesis, NfL at admission represented the mainstay of included values in our meta-analysis, which might explain our finding of the strong association between the marker and survival (**Smeele Brain comm 2022**).

Nevertheless, the general prognostic value of NfL in our cohort was too limited to be used as a standalone predictive parameter in the clinical setting. In detail, at least in mortality prediction, the accuracy of NfL Z-score alone was still insufficient to provide meaningful and univocal information with the cut-offs set. Indeed, despite reaching an overall 72% accuracy, the critical issue of low sensitivity at non-absolute thresholds for specificity is sufficient to discourage attempts to promote the single biomarker as the main driver of prognosis, particularly as this concept may have detrimental implications on hospital care. On the other hand, blood NfL might well represent a complementary, rapid and robust test for a multimodal assessment to simplify the early approach to hospitalised COVID-19 patients. Conversely, in patients after OHCA, blood NfL showed a higher prognostic value compared to those of other classical investigations, such as blood markers (neuron-specific enolase, NSE, and S100b), head computed tomography and electro-encephalography (**Paper Jama AC, Abu Rumeileh 2022**). Nevertheless, this represents a topic which is still largely unexplored in COVID-19 and deserves further investigations in large and longitudinal cohorts. Here, it might be also of interest to investigate

whether single or repeated measurements with trends over time might further improve the sensitivity of the biomarker to predict clinical outcomes.

In addition, our study also replicates, in a very large cohort of adult patients, the potential influence of several physiological and pathophysiological factors on blood NfL. The strong correlation between blood NfL and age is well-known in literature, and it is possibly related to ageing and age-related comorbidities (**Khalil Nature comm 2020, Abu Rumeileh 2022**). Therefore, the adoption of NfL Z-scores instead of raw biomarker concentrations (**Benkert et al., Lancet Neurology, 2022**) allowed to add robustness to our findings, overcoming the potential lower consistency of unadjusted analyses. As previously described (**Fitzgerald 2022**, **Rubsamen 2022, Akamine 2020, Abu-Rumeileh 2022, Hay 2021**), renal dysfunction, hypertension and diabetes also influenced blood NfL variability in our cohort.

The major strength of our study relies on the generalisation of findings derived from small, heterogeneous and geographically distinct cohorts to the population at large. This feature might allow to overcome a potential single-centre bias and to optimise the diagnostic-prognostic assessment and standard care in COVID-19 patients. On the other side, given the high heterogeneity of clinical groups recruited among the centres, biomarker median levels resulted strongly variable across distinct centres. Moreover, many of the variables considered for the analyses did not have complete data as the present meta-analysis was not pre-specified.

The present IPD meta-analysis showed that blood NfL, although showing a significant association with main clinical outcomes, should not be considered as a single parameter for routine purpose in hospitalised patients with COVID-19, due to its fair prognostic performance. Further studies should evaluate whether combining the prognostic value of NfL with that of other biological and clinical markers of COVID-19 severity may allow to create reliable scores of easy implementations for outcomes prognostication in clinical practice.

Data availability statement: The complete dataset used for this IPD will be shared with all qualified researchers upon request to the corresponding authors and after approval from participating centres who provided data.

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Competing interests: M.O. gave scientific advice to Axon, Biogen Idec, Fujirebio and Roche, all unrelated to the work presented in this paper. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). The other authors report no financial disclosures.

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REFERENCES

1. Aamodt AH, Høgestøl EA, Popperud TH, Holter JC, Dyrhol-Riise AM, Tonby K, Stiksrud B, Quist-Paulsen E, Berge T, Barratt-Due A, Aukrust P, Heggelund L, Blennow K, Zetterberg H, Harbo HF. Blood neurofilament light concentration at admittance: a potential prognostic marker in COVID-19. J Neurol. 2021 Oct;268(10):3574-3583. doi: 10.1007/s00415-021-10517-6. Epub 2021 Mar 20. PMID: 33743046; PMCID: PMC7980743.
2. Abdelhak A, Foschi M, Abu-Rumeileh S, Yue JK, D'Anna L, Huss A, Oeckl P, Ludolph AC, Kuhle J, Petzold A, Manley GT, Green AJ, Otto M, Tumani H. Blood GFAP as an emerging biomarker in brain and spinal cord disorders. Nat Rev Neurol. 2022 Mar;18(3):158-172. doi: 10.1038/s41582-021-00616-3. Epub 2022 Feb 3. PMID: 35115728.
3. Abu-Rumeileh S, Abdelhak A, Foschi M, D'Anna L, Russo M, Steinacker P, Kuhle J, Tumani H, Blennow K, Otto M. The multifaceted role of neurofilament light chain protein in non-primary neurological diseases. Brain. 2022 Sep 9:awac328. doi: 10.1093/brain/awac328.
4. Akamine S, Marutani N, Kanayama D, et al. Renal function is associated with blood neurofilament light chain level in older adults. Scientific Reports. 2020;10(1):20350. doi:10.1038/s41598-020-76990-7
5. Ameres M, Brandstetter S, Toncheva AA, et al. Association of neuronal injury blood marker neurofilament light chain with mild-to-moderate COVID-19. Journal of Neurology. 2020;267(12). doi:10.1007/s00415-020-10050-y
6. Baig AM, Khaleeq A, Ali U, Syeda H. Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host–Virus Interaction, and Proposed Neurotropic Mechanisms. ACS Chemical Neuroscience. 2020;11(7). doi:10.1021/acschemneuro.0c00122
7. Benkert P, Meier S, Schaedelin S, et al. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. The Lancet Neurology. 2022;21(3):246-257. doi:10.1016/S1474-4422(22)00009-6
8. Bornhorst JA, Figdore D, Campbell MR, Pazdernik VK, Mielke MM, Petersen RC, Algeciras-Schimnich A. Plasma neurofilament light chain (NfL) reference interval determination in an Age-stratified cognitively unimpaired cohort. Clin Chim Acta. 2022 Aug 27;535:153-156. doi: 10.1016/j.cca.2022.08.017. Epub ahead of print. PMID: 36041549.

9. Cooper J, Stukas S, Hoiland RL, et al. Quantification of Neurological Blood-Based Biomarkers in Critically Ill Patients With Coronavirus Disease 2019. Critical Care Explorations. 2020;2(10). doi:10.1097/CCE.0000000000000238
10. de Lorenzo R, Loré NI, Finardi A, et al. Blood neurofilament light chain and total tau levels at admission predict death in COVID-19 patients. Journal of Neurology. Published online May 10, 2021. doi:10.1007/s00415-021-10595-6
11. De Lorenzo R, Loré NI, Finardi A, Mandelli A, Cirillo DM, Tresoldi C, Benedetti F, Ciceri F, Rovere-Querini P, Comi G, Filippi M, Manfredi AA, Furlan R. Blood neurofilament light chain and total tau levels at admission predict death in COVID-19 patients. J Neurol. 2021 Dec;268(12):4436-4442. doi: 10.1007/s00415-021-10595-6. Epub 2021 May 10. PMID: 33973106; PMCID: PMC8108733.
12. Ehler J, Petzold A, Wittstock M, Kolbaske S, Gloger M, Henschel J, Heslegrave A, Zetterberg H, Lunn MP, Rommer PS, Grossmann A, Sharshar T, Richter G, Nöldge-Schomburg G, Sauer M. The prognostic value of neurofilament levels in patients with sepsis-associated encephalopathy - A prospective, pilot observational study. PLoS One. 2019 Jan 24;14(1):e0211184. doi: 10.1371/journal.pone.0211184. Erratum in: PLoS One. 2019 Feb 20;14(2):e0212830. PMID: 30677080; PMCID: PMC6345472.
13. Ellul MA, Benjamin L, Singh B, et al. Neurological associations of COVID-19. The Lancet Neurology. 2020;19(9). doi:10.1016/S1474-4422(20)30221-0
14. Fällmar D, Rostami E, Kumlien E, Ashton NJ, Jackmann S, Pavel R, Blennow K, Hultström M, Lipcsey M, Frithiof R, Westman G, Zetterberg H, Wikström J, Virhammar J. The extent of neuroradiological findings in COVID-19 shows correlation with blood biomarkers, Glasgow coma scale score and days in intensive care. J Neuroradiol. 2022 Nov;49(6):421-427. doi: 10.1016/j.neurad.2021.11.003. Epub 2021 Nov 18. PMID: 34800562; PMCID: PMC8599173.
15. Fitzgerald KC, Sotirchos ES, Smith MD, et al. Contributors to serum NfL levels in people without neurologic disease. Annals of Neurology. Published online June 21, 2022. doi:10.1002/ana.26446
16. Guan W jie, Ni Z yi, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. New England Journal of Medicine. 2020;382(18). doi:10.1056/NEJMoa2002032
17. Harp C, Thanei GA, Jia X, Kuhle J, Leppert D, Schaedelin S, Benkert P, von Büdingen HC, Hendricks R, Herman A. Development of an age-adjusted model for blood neurofilament light chain. Ann Clin Transl Neurol. 2022 Apr;9(4):444-453. doi: 10.1002/acn3.51524. Epub 2022 Mar 1. PMID: 35229997; PMCID: PMC8994974.
18. Hay M, Ryan L, Huentelman M, Konhilas J, Hoyer-Kimura C, Beach TG, Serrano GE, Reiman EM, Blennow K, Zetterberg H, Parthasarathy S. Serum Neurofilament Light is elevated in COVID-19 Positive Adults in the ICU and is associated with Co-Morbid Cardiovascular Disease, Neurological Complications, and Acuity of Illness. Cardiol Cardiovasc Med. 2021 Oct;5(5):551-565. doi: 10.26502/fccm.92920221. Epub 2021 Oct 13. PMID: 34708189; PMCID: PMC8547787.

19. Hoiland RL, Rikhraj KJK, Thiara S, Fordyce C, Kramer AH, Skrifvars MB, Wellington CL, Griesdale DE, Fergusson NA, Sekhon MS. Neurologic Prognostication After Cardiac Arrest Using Brain Biomarkers: A Systematic Review and Meta-analysis. JAMA Neurol. 2022 Apr 1;79(4):390-398. doi: 10.1001/jamaneurol.2021.5598. PMID: 35226054; PMCID: PMC8886448.
20. Hviid CVB, Knudsen CS, Parkner T. Reference interval and preanalytical properties of serum neurofilament light chain in Scandinavian adults. Scandinavian Journal of Clinical and Laboratory Investigation. 2020;80(4):291-295. doi:10.1080/00365513.2020.1730434
21. Izcovich A, Ragusa MA, Tortosa F, Marzio MAL, Agnoletti C, Bengolea A, Ceirano A, Espinosa F, Saavedra E, Sanguine V, Tassara A, Cid C, Catalano HN, Agarwal A, Foroutan F, Rada G. Correction: Prognostic factors for severity and mortality in patients infected with COVID-19: A systematic review. PLoS One. 2022 May 26;17(5):e0269291. doi: 10.1371/journal.pone.0269291. Erratum for: PLoS One. 2020 Nov 17;15(11):e0241955. PMID: 35617192; PMCID: PMC9135219.
22. Kanberg N, Ashton NJ, Andersson LM, et al. Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19. Neurology. 2020;95(12). doi:10.1212/WNL.0000000000010111
23. Kanberg N, Simrén J, Edén A, et al. Neurochemical signs of astrocytic and neuronal injury in acute COVID-19 normalizes during long-term follow-up. EBioMedicine. 2021;70. doi:10.1016/j.ebiom.2021.103512
24. Khalil M, Pirpamer L, Hofer E, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. Nature Communications. 2020;11(1). doi:10.1038/s41467-020-14612-6
25. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. Nature Reviews Neurology. 2018;14(10). doi:10.1038/s41582-018-0058-z
26. Li Y, Bai W, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. Journal of Medical Virology. 2020;92(6). doi:10.1002/jmv.25728
27. Luchini C, Stubbs B, Solmi M, et al. Assessing the quality of studies in meta analyses: advantages and limitations of the Newcastle Ottawa Scale. World J Meta Analysis 2017; 5: 80.
28. Mao L, Jin H, Wang M, et al. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. JAMA Neurology. 2020;77(6). doi:10.1001/jamaneurol.2020.1127
29. Mariotto S, Savoldi A, Donadello K, et al. Nervous system: subclinical target of SARS-CoV-2 infection. Journal of Neurology, Neurosurgery & Psychiatry. 2020;91(9). doi:10.1136/jnnp-2020-323881
30. Masvekar RR, Kosa P, Jin K, Dobbs K, Stack MA, Castagnoli R, Quaresima V, Su HC, Imberti L, Notarangelo LD, Bielekova B. Prognostic value of serum/plasma neurofilament light chain for COVID-19-associated mortality. Ann Clin Transl Neurol. 2022 May;9(5):622-632. doi: 10.1002/acn3.51542. Epub 2022 Mar 21. PMID: 35313387; PMCID: PMC9082006.

31. Needham EJ, Ren AL, Digby RJ, Norton EJ, Ebrahimi S, Outtrim JG, Chatfield DA, Manktelow AE, Leibowitz MM, Newcombe VFJ, Doffinger R, Barcenas-Morales G, Fonseca C, Taussig MJ, Burnstein RM, Samanta RJ, Dunai C, Sithole N, Ashton NJ, Zetterberg H, Gisslén M, Edén A, Marklund E, Openshaw PJM, Dunning J, Griffiths MJ, Cavanagh J, Breen G, Irani SR, Elmer A, Kingston N, Summers C, Bradley JR, Taams LS, Michael BD, Bullmore ET, Smith KGC, Lyons PA, Coles AJ, Menon DK; Cambridge NeuroCOVID Group; CITIID-NIHR COVID-19 BioResource Collaboration; Cambridge NIHR Clinical Research Facility. Brain injury in COVID-19 is associated with dysregulated innate and adaptive immune responses. Brain. 2022 Nov 21;145(11):4097-4107. doi: 10.1093/brain/awac321. PMID: 36065116; PMCID: PMC9494359.
32. Pezzini A, Padovani A. Lifting the mask on neurological manifestations of COVID-19. Nature Reviews Neurology. 2020;16(11). doi:10.1038/s41582-020-0398-3106.
33. Polymeris AA, Helfenstein F, Benkert P, et al. Renal Function and Body Mass Index Contribute to Serum Neurofilament Light Chain Levels in Elderly Patients With Atrial Fibrillation. Frontiers in Neuroscience. 2022;16. doi:10.3389/fnins.2022.819010
34. Prudencio M, Erben Y, Marquez CP, Jansen-West KR, Franco-Mesa C, Heckman MG, White LJ, Dunmore JA, Cook CN, Lilley MT, Song Y, Harlow CF, Oskarsson B, Nicholson KA, Wszolek ZK, Hickson LJ, O'Horo JC, Hoyne JB, Gendron TF, Meschia JF, Petrucelli L. Serum neurofilament light protein correlates with unfavorable clinical outcomes in hospitalized patients with COVID-19. Sci Transl Med. 2021 Jul 14;13(602):eabi7643. doi: 10.1126/scitranslmed.abi7643. Epub 2021 Jun 15. PMID: 34131052; PMCID: PMC8432951.
35. Rübsamen N, Willemse EAJ, Leppert D, Wiendl H, Nauck M, Karch A, Kuhle J, Berger K. A Method to Combine Neurofilament Light Measurements From Blood Serum and Plasma in Clinical and Population-Based Studies. Front Neurol. 2022 Jun 14;13:894119. doi: 10.3389/fneur.2022.894119. PMID: 35775045; PMCID: PMC9237479.
36. Simrén J, Andreasson U, Gobom J, et al. Establishment of reference values for plasma neurofilament light based on healthy individuals aged 5-90 years. Brain Communications. Published online July 4, 2022. doi:10.1093/braincomms/fcac174
37. Smeele PJ, Vermunt L, Blok S, Duitman JW; AmsterdamUMC COVID-19 Biobank; Nossent EJ, van Agtmael MA, Heunks LMA, Horn J, Bogaard HJ, Teunissen CE. Neurofilament light increases over time in severe COVID-19 and is associated with delirium. Brain Commun. 2022 Jul 26;4(4):fcac195. doi: 10.1093/braincomms/fcac195. PMID: 35938070; PMCID: PMC9351727.
38. Stewart LA, Clarke M, Rovers M, Riley RD, Simmonds M, Stewart G, Tierney JF; PRISMA-IPD Development Group. Preferred Reporting Items for Systematic Review and Meta-Analyses of individual participant data: the PRISMA-IPD Statement. JAMA. 2015 Apr 28;313(16):1657-65. doi: 10.1001/jama.2015.3656. PMID: 25919529.
39. Sutter R, Hert L, De Marchis GM, Twerenbold R, Kappos L, Naegelin Y, Kuster GM, Benkert P, Jost J, Maceski AM, Rüegg S, Siegemund M, Leppert D, Tschudin-Sutter S, Kuhle J. Serum Neurofilament

Light Chain Levels in the Intensive Care Unit: Comparison between Severely Ill Patients with and without Coronavirus Disease 2019. Ann Neurol. 2021 Mar;89(3):610-616. doi: 10.1002/ana.26004. Epub 2021 Jan 11. PMID: 33377539.

40. Virhammar J, Nääs A, Fällmar D, Cunningham JL, Klang A, Ashton NJ, Jackmann S, Westman G, Frithiof R, Blennow K, Zetterberg H, Kumlien E, Rostami E. Biomarkers for central nervous system injury in cerebrospinal fluid are elevated in COVID-19 and associated with neurological symptoms and disease severity. Eur J Neurol. 2021 Oct;28(10):3324-3331. doi: 10.1111/ene.14703.
41. World Health Organization. Clinical Management of COVID-19: Interim Guidance. World Health Organization; 2020:13–15.

SUPPLEMENTARY MATERIALS

Box 1. Search string
["blood biomarker" OR "serum biomarker" OR "plasma biomarker" OR "blood neurofilament light chain" OR "blood NfL" OR "serum neurofilament light chain" OR "serum NfL" OR "plasma neurofilament light chain" OR "plasma NfL" OR "neurofilament" OR "neurofilament light chain" OR "NfL"]) AND (["COVID-19" OR "Wuhan coronavirus" OR "novel coronavirus" OR "novel coronavirus 2019" OR "SARS" OR "SARS-CoV-2"])

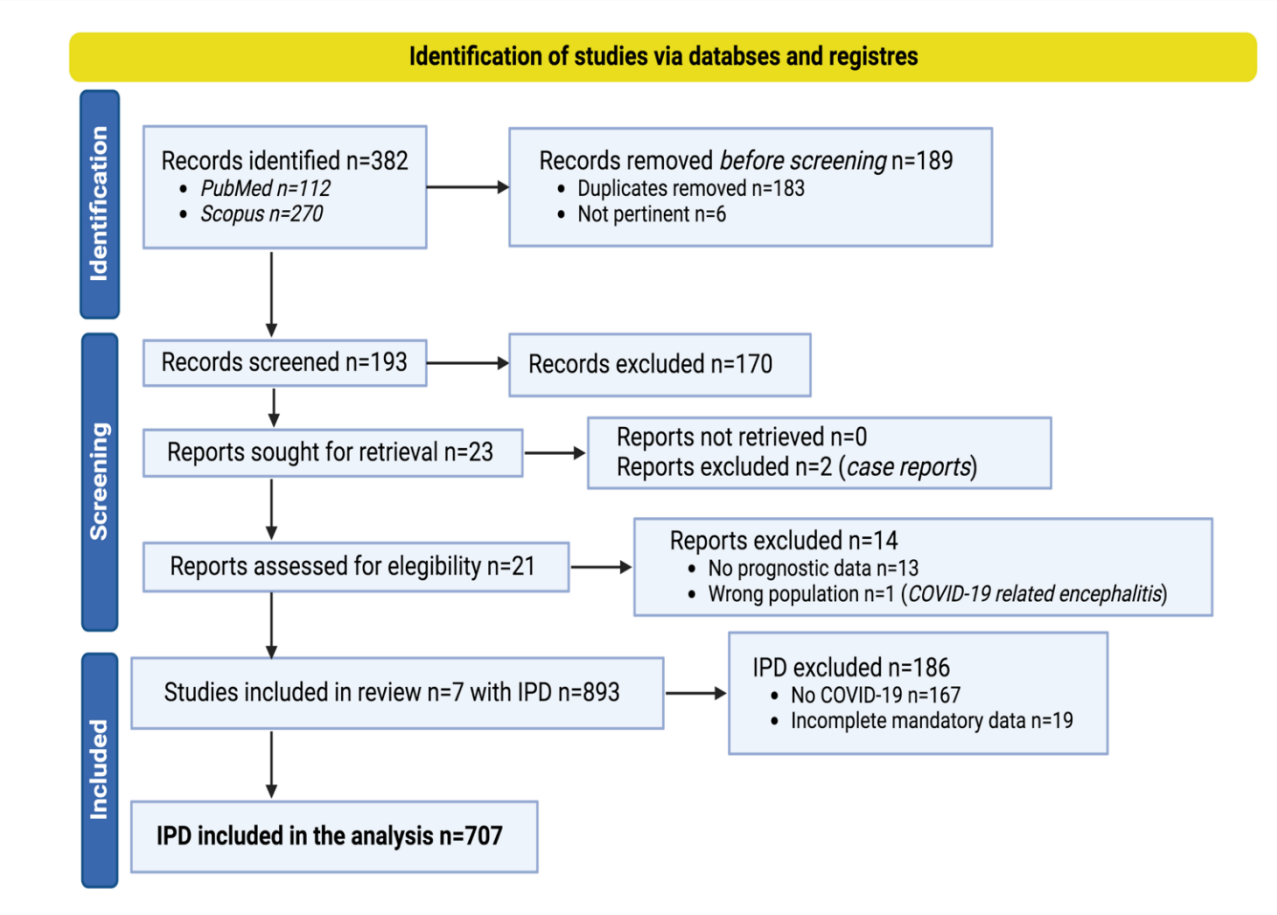
Supplementary Table 1. Number of individual participant data (IPD) included in the meta-analysis, analytical and pre-analytical features of the 7 studies.

Authors (year)	**N of IPD**	**Center (Country)**	**Sample matrix**	**Assay**	**Kit**
Aamodt et al. (2021)	46	Oslo, Drammen (Norway)	Serum	Simoa	Neurology 4-Plex A
De Lorenzo et al. (2021)	104	Milan (Italy)	Plasma	Simoa	Neurology 4-Plex B
Fällmar et al. (2021)*	19	Uppsala (Sweden)	Plasma	Simoa	Neurology 4-Plex A
Masvekar et al. (2022)	370	Brescia (Italy)	Plasma	Simoa	NF-LIGHT
Prudencio et al. (2021)	142	Jacksonville (USA)	Serum	Simoa	NF-LIGHT
Sutter et al. (2021)	26	Basel (Switzerland)	Serum	Simoa	NF-LIGHT

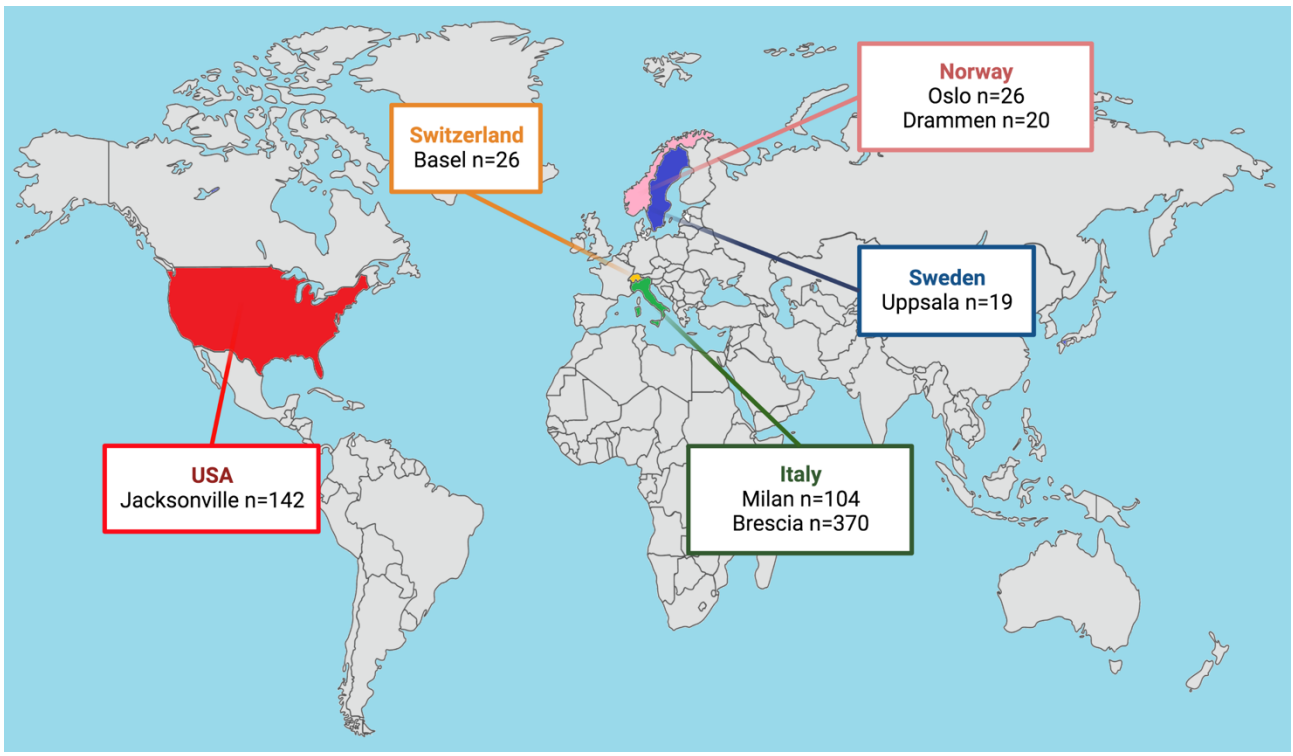
Virhammar et al. (2020)*	14	Uppsala (Sweden)	Plasma	Simoa	Neurology 4-Plex A

*n=14/19 overlapping IPD.

Supplementary figure 1. PRISMA flow-chart. IPD = individual patient data.



Supplementary figure 2. Location of the 7 recruiting centers providing individual participant data (IPD). Country names and number of patients for which IPD were analyzed are displayed in boxes. Figure created with Biorender.com.



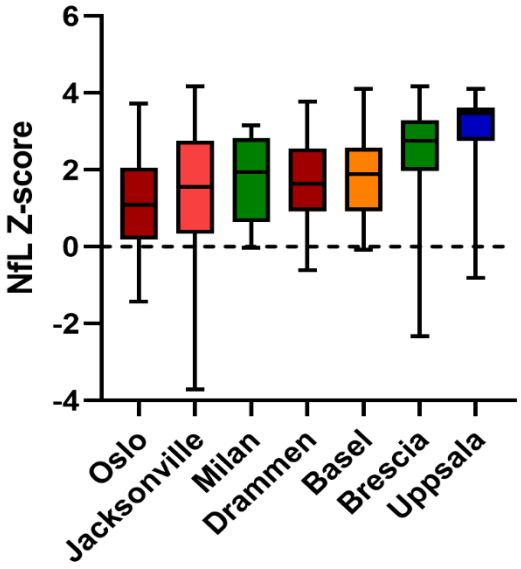
Supplementary Table 2. Demographical, clinical and laboratory features of COVID-19 patients per recruiting center.

	Oslo (n=26)		Drammen (n=20)		Milan (n=104)		Uppsala (n=19)		Brescia (n=370)		Basel (n=26)		Jacksonville (n=142)	
	value	n. cases	value	n. cases	value	n. cases	Value	n. cases	Value	n. cases	value	n. cases	value	n. cases
Sex (female/male) [frequency (%)]	9 (34.6) / 17 (65.4)	26	-	-	40 (38.5) / 64 (61.5)	104	7 (36.8) / 12 (63.2)	19	94 (25.4) / 276 (74.6)	370	7 (26.9) / 19 (73.1)	26	60 (42.3) / 82 (57.7)	142
Age [mean (± sd) (range)]	59.6 (± 15.0) (30.0 - 87.0)	26	60.5 (± 18.2) (27 - 93)	20	57.8 (48.5 - 67.0) (27.9 - 84.7)	104	64 (48 - 72) (34 - 76)	19	73.5 (66.0 - 80.3) (32.5 - 99.1)	370	65 (55 - 70) (37 - 92)	26	62 (48 - 72) (22 - 99)	142
NfL raw values (pg/ml) [median (IQR) (range)]	17.1 (9.9 - 30.2) (5.8 - 174.4)	26	26.4 (9.0 - 46.9) (6.5 - 122.7)	20	18.1 (12.6 - 35.1) (5.6 - 261.3)	104	97.7 (43.1 - 180.9) (5.86 - 877.0)	19	53.6 (28.8 - 105.5) (5.6 - 2233.2)	370	21.8 (17.1 - 40.3) (7.5 - 1311.7)	26	21.8 (11.1 - 49.0) (2.9 - 1538.5)	142
NfL log-transformed values (pg/ml) [median (IQR) (range)]	1.23 (1.00 - 1.48) (0.76 2.24)	26	1.42 (0.95 - 1.64) (0.81 - 2.09)	20	1.26 (1.10 - 1.55) 0.75 - 2.42)	104	1.99 (1.63 - 2.26) (0.77 - 2.94)	19	1.73 (1.46 - 20.2) (0.75 - 3.35)	370	1.34 (1.23 - 1.61) (0.88 - 3.12)	26	1.34 (1.05 - 1.69) (0.46 - 3.19)	142
NfL Z-scores [median (IQR) (range)]	1.09 (0.22 - 2.00) (-1.44 - 3.72)	26	1.94 (0.67 - 2.77) (-0.03 - 3.16)	20	1.64 (0.92 - 2.49) (-0.61 - 3.78)	104	3.48 (2.88 - 3.62) (-0.81 - 4.11)	19	2.75 (1.98 - 3.29) (-2.33 - 4.17)	370	1.90 (1.02 - 2.56) (-0.08 - 4.11)	26	1.55 (0.37 - 2.72) (-3.72 - 4.17)	142
Current disease														

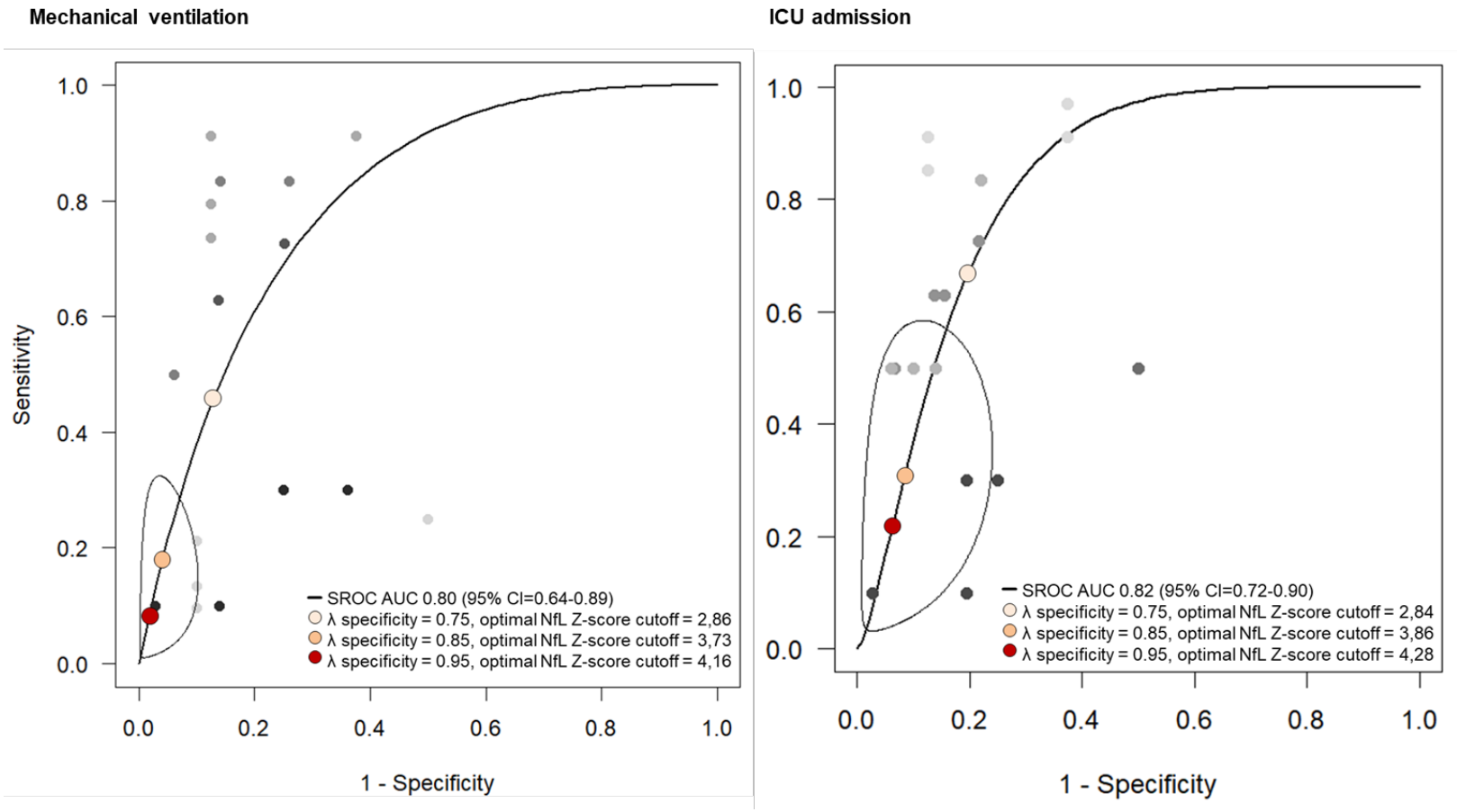
Time to admission (days) [median (IQR)]	8.0 (5.5 - 11.8)	26	7.0 (6.0-7.8)	4	7 (4 - 10)	104	-	-	5 (3 - 8)	130	-	-	-	-
Time to blood sampling (days) [median (IQR)]	8.0 (5.5 - 11.8)	26	7.0 (6.0-7.8)	4	8 (5 - 11)	104	24 (21 - 30)	19	14 (8 - 19)	127	-	-	1 (0 - 3)	142
COVID-19 severity (moderate/severe/critical) [frequency (%)]	-	-	-	-	-	-	4 (4 -4)	19	4 (4 - 4)	370	3 (3 - 3)	26	4 (4 - 4)	54
Comorbidities														
Diabetes (yes/no) [frequency (%)]	-	-	-	-	21 (20.2%) / 83 (79.8%)	104	5 (26.3) / 14 (73.7)	19	36 (12.6%) / 250 (87.4%)	286	-	-	-	-
Hypertension (yes/no) [frequency (%)]	9 (52.9) / 8 (47.1)	17	-	-	38 (36.5%) / 66 (63.5%)	104	10 (52.6) / 9 (47.4)	19	56 (19.6%) / 230 (80.4%)	286	-	-	-	-
Laboratory analyses														
Lymphocyte count (x10^9/l) [median (IQR)]	-	-	-	-	1.00 (0.80 - 1.30)	98	8.1 (5.2 - 13.9)	15	0.85 (0.51 - 1.17)	146	0.47 (0.27 - 0.67)	26	-	-
Neutrophil count (x10^9/l) [median (IQR)]	3.9 (2.4 - 6.2)	26	4.3 (3.0 - 7.1)	20	5.3 (3.7 - 8.0)	93	7.5 (5.5 - 10.2)	13	-	-	-	-	-	-
CRP (mg/l) [median (IQR)]	48.5 (10.5 - 124.5)	26	76.0 (52.0 - 157.5)	20	89.2 (24.7 - 152.0)	104	58.0 (11.1 - 135.5)	15	34.2 (9.1 - 98.4)	147	181.4 (126.9 - 255.1)	26	-	-
LDH (U/l) [median (IQR)]	-	-	-	-	387 (274 - 520)	101	-	-	309 (267 - 424)	146	-	-	-	-

Creatinin (mg/dl) [median (IQR)]	0.80 (0.69 - 0.87)	25	0.80 (0.63 - 1.30)	20	0.88 (0.72 - 1.13)	104	-	-	-	-	-	-	-	-
PaO2/FiO2 [median (IQR)]	-	-	-	-	284 (191 - 367)	101	71.3 (65.3 - 96.8)	17	-	-	157 (107 - 177)	23	-	-
Outcomes														
Days of hospitalization [median (IQR)]	1 (1 - 3)	25	2.5 (2.0 - 5.8)	4	10 (0 - 25)	103	49 (21 - 61)	19	27 (16 - 41)	187	22 (11 - 28)	26	9 (6 - 15)	142
Mechanical ventilation (yes/no) [frequency (%)]	2 (7.7) / 24 (92.3)	26	4 (20.0) / 16 (80.0)	20	-	-	16 (84.2) / 3 (15.8)	19	-	-	22 (84.6) / 4 (15.4)	26	30 (21.1) / 112 (78.9)	142
Days of mechanical ventilation [median (IQR)]	-	-	-	-	-	-	-	-	-	-	10 (6 - 18)	22	3 (0 - 14)	30
ICU admission (yes/no) [frequency (%)]	6 (23.1) / 20 (76.9)	26	5 (25.0) / 15 (75.0)	20	34 (32.7) / 70 (67.3)	104	17 (89.5) / 2 (10.5)	19	129 (41.6) / 181 (58.4)	310	26 (100.0) / 0 (0.0)	26	54 (38.0) / 88 (62.0)	142
Death (yes/no) [frequency (%)]	2 (7.7) / 24 (92.3)	26	4 (20.0) / 16 (80.0)	20	22 (21.2) / 82 (78.8)	104	2 (10.5) / 17 (89.5)	19	150 (40.5) / 220 (59.5)	370	5 (19.2) / 21 (80.8)	26	13 (9.2) / 129 (90.8)	142

Supplementary figure 3. Distribution of NfL Z-scores median values across recruiting centres



Supplementary Figure 4. Summary ROC for NfL Z-score prediction of mechanical ventilation (left) and admission to ICU (right) according to predefined specificity thresholds.



Mechanical ventilation. Specificity 95% optimal cut-off 4.16 (sensitivity 0.08, specificity 0.98), specificity 85% optimal cut-off 3.73 (sensitivity 0.17, specificity 0.96), specificity 75% optimal cut-off 2.86 (sensitivity 0.49, specificity 0.86). At maximized Youden-Index optimal cut-off 2.03 (sensitivity 0.80, specificity 0.66)

ICU admission. Specificity 95% optimal cut-off 4.28 (sensitivity 0.21, specificity 0.94), specificity 85% optimal cut-off 3.86 (sensitivity 0.32, specificity 0.91), specificity 75% optimal cut-off 84 (sensitivity 0.67, specificity 0.80). At maximized Youden-Index optimal cut-off 2.00 (sensitivity 0.88, specificity 0.67)