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Systemic inflammation relates to neuroaxonal damage associated with long-term cognitive dysfunction in COVID-19 patients

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ABSTRACT

Background and objectives: Cognitive deficits are increasingly recognized as a long-term sequela of severe COVID-19. The underlying processes and molecular signatures associated with these long-term neurological sequalae of COVID-19 remain largely unclear, but may be related to systemic inflammation-induced effects on the brain. We studied the systemic inflammation-brain interplay and its relation to development of long-term cognitive impairment in patients who survived severe COVID-19. Trajectories of systemic inflammation and neuroaxonal damage blood biomarkers during ICU admission were analyzed and related to long-term cognitive outcomes.

Methods: Prospective longitudinal cohort study of patients with severe COVID-19 surviving ICU admission. During admission, blood was sampled consecutively to assess levels of inflammatory cytokines and neurofilament light chain (NfL) using an ultrasensitive multiplex Luminex assay and single molecule array technique (Simoa). Cognitive functioning was evaluated using a comprehensive neuropsychological assessment six months after ICU-discharge.

Results: Ninety-six patients (median [IQR] age 61 [55–69] years) were enrolled from March 2020 to June 2021 and divided into two cohorts: those who received no COVID-19-related immunotherapy (n = 28) and those treated with either dexamethasone or dexamethasone and tocilizumab (n = 68). Plasma NfL concentrations increased in 95 % of patients during their ICU stay, from median [IQR] 23 [18–38] pg/mL at admission to 250 [160–271] pg/mL after 28 days, $p < 0.001$. Besides age, glomerular filtration rate, immunomodulatory treatment, and C-reactive protein, more specific markers of systemic inflammation at day 14 (i.e., interleukin (IL)-8, tumour necrosis factor, and IL-1 receptor antagonist) were significant predictors of blood NfL levels at day 14 of ICU admission ($R^2 = 44$ %, $p < 0.001$), illustrating the association between sustained systemic inflammation and neuroaxonal damage. Twenty-six patients (27 %) exhibited cognitive impairment six months after discharge from the ICU. NfL concentrations showed a more pronounced increase in patients that developed cognitive impairment ($p = 0.03$). Higher NfL predicted poorer outcome in information processing speed (Trail Making Test A, $r = -0.26$, $p = 0.01$; Letter Digit Substitution Test, $r = -0.24$, $p = 0.02$).

Discussion: Prolonged systemic inflammation in critically ill COVID-19 patients is related to neuroaxonal damage and subsequent long-term cognitive impairment. Moreover, our findings suggest that plasma NfL concentrations during ICU stay may possess prognostic value in predicting future long-term cognitive impairment in patients that survived severe COVID-19.

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1. Introduction

Coronavirus disease 2019 (COVID-19) is associated with a broad spectrum of neurological long-term consequences in a subgroup of patients (Taquet et al., 2021; Peter et al., 2022), which may lead to significant long-term impairment in both occupational and daily activities (Davis et al., 2021). Several mechanisms are implicated to play a role in these neurological sequelae observed in severe cases of COVID-19, including hypoxemia or hypotension-related damage, microhaemorrhages, and ischemic strokes as shown in observational neuroimaging studies (Manca et al., 2021). Furthermore, neuropathological studies showed diffuse neuroinflammatory lesions in the brain (Matschke et al., 2020; Schurink et al., 2020), indicating that neuroinflammation may play an important role. This was recently supported by an *in vivo* quantitative assessment of neuroinflammation in patients with long-COVID (Braga et al., 2023). The significance of neuroinflammation in COVID-19 is further demonstrated by biomarker studies conducted on plasma and cerebrospinal fluid (CSF) samples from COVID-19 patients, showing evidence of neuroaxonal damage (Kanberg et al., 2020; Virhammar et al., 2020; Edén et al., 2021). Notably, increased levels of neurofilament light (NfL), a neuronal cytoskeletal protein that can be detected in both CSF and plasma and reflects neuroaxonal damage (Khalil et al., 2018), were found to correlate with disease severity (Kanberg et al., 2020; Virhammar et al., 2020; Aamodt et al., 2021). Moreover, NfL levels predicted encephalopathy, other neurological symptoms, and mortality in hospitalized COVID-19 patients (Virhammar et al., 2020; De Lorenzo et al., 2021; Frontera et al., 2022; Marchegiani et al., 2023; Masvekar et al., 2022; Plantone et al., 2022; Prudencio et al., 2021).

Systemic inflammation is hypothesized to induce neuroinflammation in COVID-19 patients [19, 20)]. Although the relationship between systemic inflammation and markers for neuroaxonal damage was studied before, previous work often only performed sampling at a single timepoint (Virhammar et al., 2020; Aamodt et al., 2021; De Lorenzo et al., 2021; Frontera et al., 2022; Marchegiani et al., 2023; Plantone et al., 2022; Prudencio et al., 2021; Ameres et al., 2020; de Boni et al., 2022; Hanson et al., 2022; Hay et al., 2021; Paterson et al., 2021 ; Sahin et al., 2023; Sun et al., 2021). In addition, longitudinal neuropsychological follow-up in such studies is rare and assessment of cognitive consequences is often limited to self-report questionnaires (Hanson et al., 2022; Sahin et al., 2023; Guasp et al., 2022; Kanberg et al., 2021; Needham et al., 2022; Peluso et al., 2022). Also, the impact of COVID-19 related immunomodulatory therapies such as dexamethasone or IL-6 receptor antagonists has not been investigated previously (Bonetto et al., 2022; Smeele et al., 2022).

The two main aims of this study were to analyse (i) the trajectories of blood markers of systemic inflammation and neuroaxonal damage (NfL), and (ii) how these relate to cognitive outcomes based on extensive neuropsychological assessments six months after discharge in a cohort of patients who survived severe COVID-19. As immunomodulatory therapies for COVID-19 were implemented *during* the inclusion period of this cohort, an additional analysis was conducted to assess whether these treatments influenced NfL trajectories.

2. Methods

2.1. Study design and population

This is a single-center longitudinal cohort study, performed at Radboud university medical center in Nijmegen, The Netherlands. We screened all adult patients admitted to the intensive care unit (ICU) with COVID-19 confirmed through RT-PCR analysis of nasal and throat swab specimens, between March 18, 2020 and June 6, 2021 (main SARS-CoV-2 variant during that period was Alpha (B.1.1.7.) (RIVM, 2022). As our primary outcome was a detailed neuropsychological assessment at 6 months post-hospitalization, only survivors up to 6 months were included in this study.

During the pandemic, dexamethasone and the IL-6 receptor antagonist tocilizumab were introduced as standard of care in patients with severe COVID-19 infection (Horby et al., 2021; Investigators et al., 2021). Therefore, two cohorts were established. The first cohort consists of patients from the first COVID-19 surge admitted between March 18, 2020 and April 27, 2020, who did not receive any immunomodulatory therapy. The second cohort comprises patients from subsequent COVID-19 surges admitted between October 6, 2020 and June 6, 2021, who received either dexamethasone (once daily 6 mg intravenous for ten consecutive days) or dexamethasone and tocilizumab (single intravenous dose 400–800 mg, 8 mg/kg).

Demographic and ICU-specific information was collected, including sex, age, medical history, symptom onset, date of admission to ICU, disease severity, and mechanical ventilation. None of the patients had psychiatric or neurological comorbidities at ICU admission. Glomerular filtration rate (GFR) was calculated using the MDRD formula (Levey et al., 2006).

2.2. Blood sampling and analyses

Consecutive blood sampling was conducted every other day during their ICU-length of stay, starting from day 1 (within the first 24 h following ICU admission) and continued until ICU discharge for a maximum of 28 days. If patients had previously been admitted to another ICU before transferring to the academic hospital, the first available sample was used. Blood samples were collected in ethylenediamine tetra-acetic acid (EDTA) tubes and centrifuged (2000g, 10 min, 4 °C), after which plasma was stored at −80 °C until further analysis. Concentrations of interleukin (IL)-6, IL-8, IL-10, tumour necrosis factor (TNF), IFN-γ-induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1, and IL-1 receptor antagonist (IL-1RA) were determined using a multiplex Luminex assay (Milliplex, Millipore, Billerica, USA). The lower detection limit was 3.2 pg/mL for all cytokines. Other analytes, IL-4, IFNγ, IFNα, IFNβ, GM-CSF, IL-1a, and IL-1b, that were also assessed with the multiplex assay revealed concentrations below lower limit of quantification in $>$ 60 % of the samples and were therefore excluded from further statistical analysis. Technicians were blinded to sample identity.

Additionally, neurofilament light (NfL) concentrations were assessed in plasma samples collected at the time of admission and subsequently once every week for the remainder of ICU admission for patients from the first cohort. Plasma NfL concentrations were measured in technical duplicates, using the Simoa HD-X instrument (Quanterix, Billerica, MA, USA) and the Neurology-4-Plex or the NF-light Advantage kit following the manufacturer's instructions (dilution factor: 1 in 4 in sample buffer). All measurements had a coefficient of variation (CV) below 20 %. The lower limit of quantification (LLoQ) was 2.17 pg/ml across runs, and none of the samples had measurements below the LLoQ. Longitudinal samples were analyzed in the same batch, and technicians were blinded to sample identity.

2.3. Neuropsychological assessments and evaluation

All patients underwent a neuropsychological evaluation, administered by trained neuropsychologists, approximately six months after their discharge from the ICU. The cognitive test battery consisted of five cognitive tests, i.e. Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005), Trail Making Test (TMT) part A, and part B resulting in interference score B/A (Partington and Leiter, 1949; Reitan, 1958; Bowie and Harvey, 2006), Letter Digit Substitution Test (LDST) (Natu and Agarwal, 1995) and Digit Span (Kessels et al., 2011; Wechsler, 2012). To obtain standardized z-scores, all tests were compared to available Dutch normative data for age-, sex- and education (de Vent NR, Agelink van Rentergem JA, Schmand BA, Murre JM, Consortium A, Huizenga HM. Advanced Neuropsychological Diagnostics Infrastructure

(ANDI): A Normative Database Created from Control Datasets. Front Psychol., 2016), including the MoCA (Kessels et al., 2022). Consequently, individual test results were categorized as '*Normal*' performance (above −1 SD from normative mean), *'below average'* (between −1 SD and −1.5 SD from normative mean) and *'impaired'* (below −1.5 SD from normative mean, the lowest 6.7 % of the normal population). Cognitive performance was dichotomized as impaired when the participant classified as *'impaired'* on two or more cognitive tests (van den Berg et al., 2005; Reukers et al., 2020; Duindam et al., 2022).

2.4. Statistics

Depending on its distribution, continuous variables are presented as either mean ± standard deviation (SD) or median [first and third inter quartile range, IQR] as appropriate, and categorical variables are expressed as frequency (n) with proportions (%). Normality of data distribution was checked using Normal Q-Q-plots and Shapiro-Wilk test, and homogeneity using Levene's test. Comparisons between continuous variables in groups were made by independent samples *t*-test, Mann-Whitney-*U* test, one-way analysis of variance (ANOVA) or Kruskal-Wallis-test, as appropriate. Comparison of NfL for different treatment groups was performed using analysis of covariance (ANCOVA) with age and GFR as covariates. If statistically significant between-group differences were found, Bonferroni corrected post-hoc tests were performed.

We performed univariate correlations for cytokines and NfL using Pearson's correlation coefficients. As the assumption of normal distribution was violated for NfL and cytokine measurements, these data were log-transformed before analysis. Furthermore, multiple linear regression analyses were conducted. To prevent a surplus of variables, cytokines (and their treatment × cytokine interaction) were only added if their univariate cytokine-NfL correlation showed a p-value < 0.1. It is known that blood NfL increases during lifetime and is correlated to creatinine levels (Fitzgerald et al., 2022), so both age and glomerular filtration rate (GFR) were added as a covariate in ANCOVA and multiple linear regression models. For analysis of repeated measures, linear mixed effect (LME) models were fitted to test whether longitudinal trajectories of plasma NfL levels were associated with cognitive impairment in COVID-19 patients. The LME models were adjusted for age and GFR, and included random effects and intercepts nested within subject. Linear additivity was assessed by visual inspection of the residuals vs fitted plot. A partial correlational analysis was performed to determine the relationship between NfL and the individual neuropsychological test results, whilst also controlling for age. Alpha was set at 0.05 (two-tailed). Statistical analyses were performed using SPSS software (version 27.0, IMB Corp., Armonk, NY, USA), GraphPad Prism (version 9.5.1, LaJolla, CA, USA) and Stata (version 17.0, Stata Corp., College Station, TX, USA).

Table 1
Demographics, admission characteristics and clinical outcomes.

	First cohort	Second cohort		p-value	All patients
	D-T-	D + T-	D + T+		
N=	28	26	42		96
Demographics					
Age, years	62 [58–70]	60 [51–67]	63 [54–69]	p = 0.33	61 [55–69]
Gender, male	19 (68 %)	18 (69 %)	27 (64 %)	p = 0.90	64 (67 %)
BMI, kg/m^2	27.1 [24.4–29.2]† ‡	29.3 [27.0–32.4]*	29.4 [26.2–32.2]*	**p = 0.015**	28.3 [25.9–31.5]
Days from first COVID-19 symptoms to ICU admission	11.4 ± 4.6	11.1 ± 6.3	11.4 ± 3.1	p = 0.98	11.3 ± 4.5
Medical history, n (%)					
Hypertension	15 (54 %)	10 (39 %)	20 (48 %)	p = 0.54	45 (47 %)
Diabetes Mellitus	5 (18 %)	6 (23 %)	11 (26 %)	p = 0.72	22 (23 %)
COPD	2 (7 %)	5 (19 %)	3 (7 %)	p = 0.23	10 (10 %)
At admission to ICU					
APACHE II	15.2 ± 5.2	15.7 ± 3.9	16.2 ± 3.5	p = 0.60	15.8 ± 4.1
SOFA	6.2 ± 2.6	5.6 ± 3.0	5.6 ± 2.4	p = 0.64	5.8 ± 2.6
Temperature, °C	38.3 [37.5–38.9]† ‡	37.3 [36.7–38.0]*	37.0 [36.5–37.6]*	**p < 0.001**	37.3 [36.7–38.2]
Invasive mechanical ventilation, n (%)	28 (100 %)	26 (100 %)	33 (79 %)	**p = 0.002**	87 (91 %)
PaO2/FiO2 ratio	144 [120–189]	147 [84–206]	127 [98–162]	p = 0.30	135 [101–180]
Leukocytes, 10^9/L	8.2 [6.2–12.8]†	12.6 [9.5–15.4]* ‡	8,2 [6.5–11.1]†	**p = 0.002**	9.4 [6.7–13.0]
CRP, mg/L	190 [127–269]† ‡	75 [44–99]*	65 [27–136]*	**p < 0.001**	95 [47–171]
Ferritin, µg/L	1446 [709–2182]	1355 [741–2327]	1285 [645–1904]	p = 0.85	1345 [701–1905]
Creatinin, µmol/L	79 [63–100]	74 [60–87]	75 [55–89]	p = 0.52	75 [56–93]
GFR (MDRD), ml/min/1.73 m2	81.3 ± 32.9	85.5 ± 27.7	92.7 ± 36.6	p = 0.36	87.4 ± 33.4
Outcomes					
Time on ventilator, days	20 [12–27]‡	8 [5–27]	12 [4–19]*	**p = 0.011**	13 [6–23]
Delirium during ICU stay	22 (79 %)	19 (73 %)	29 (69 %)	p = 0.68	70 (73 %)
Delirium- and coma-free days	6 [4–11]	3 [2–6]	5 [3–8]	p = 0.098	5 [3–9]
Duration of ICU admission, days	23 [12–30]	10 [7–28]	15 [8–26]	p = 0.076	16 [9–28]

Patient groups: D-T-, no anti-inflammatory therapy; D + T-, dexamethasone, no tocilizumab; D + T+, dexamethasone and tocilizumab. Significant p-values of between-group differences were followed by Bonferroni-corrected post-hoc analyses. * $p \leq 0.05$ vs D-T-; † $p \leq 0.05$ vs D + T-; ‡ $p \leq 0.05$ vs D + T +.
Values are presented as either median [IQR], mean ± SD, or n (%).
Outcomes: Coma was assessed using the Richmond Agitation Sedation Scale (RASS) and defined as RASS ≤ -3. Delirium was assessed using the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU). Delirium- and coma-free days were defined as the number of ICU days during which the patient was without delirium and not in coma.
Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit; SD, standard deviation; IQR, interquartile range; BMI, body mass index; COPD, Chronic Obstructive Pulmonary Disease; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; PaO2, partial pressure of oxygen; FiO2, fraction of inspired oxygen; CRP, C-reactive protein; GFR (MDRD), Glomerular filtration rate (Modification of Diet in Renal Disease).

2.5. Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

3. Results

3.1. Brief description of the cohort(s)

A total of 28 patients were included in the first cohort of the study. Apart from hydroxychloroquine, which was standard of care therapy at that time, none of the patients received any immunomodulating therapy such as dexamethasone or tocilizumab (D-T-). Median age was 62 [58–70] and 19 (68 %) were male. At ICU admission, mean APACHE II score was 15.2 ± 5.2 and all patients were in need of invasive mechanical ventilation. Further baseline characteristics and clinical outcomes are listed in the first column of Table 1.

The second cohort comprised 26 patients who only received dexamethasone (D + T-; median age 60 (Duindam et al., 2022; Keske et al., 2020; Peters van Ton et al., 2021; Cunningham et al., 2009; DiSabato et al., 2016; Michelen et al., 2021; Chiaravalloti and DeLuca, 2008; Ruano et al., 2017; Kuhle et al., 2019; Gaetani et al., 2019; Neselius et al., 2014; Khalil et al., 2020; Beunders et al., 2021; Sutter et al., 2021; De Lorenzo et al., 2021; Fisse et al., 2021; Ehler et al., 2019); 69 % men) and 42 patients who received both dexamethasone and tocilizumab (D + T+; median age 63 [54–69], 64 % men). None of the patients in the second cohort received hydroxychloroquine. Compared to the first cohort (D-T-), some differences in baseline characteristics were present (see Table 1). While all patients (100 %) in the D-T- and D + T- groups required invasive mechanical ventilation, this was the case for 79 % (n = 33) of the patients in the D + T + group (p = 0.002), as high-flow nasal devices had newly been introduced as adequate respiratory support. However, all patients still met the ARDS criteria. Likely as a consequence of the treatment, body temperature at admission was lower in the immunomodulatory treatment groups (D + T- 37.3 °C [36.7–38.0] and D + T + 37.0 °C [36.5–37.6] compared to the D-T- group (38.3 °C [37.5–38.9], p < 0.001). Furthermore, patients who were treated with dexamethasone and tocilizumab had a significantly shorter time on ventilator compared to the first cohort (12 (Manca et al., 2021; Matschke et al., 2020; Schurink et al., 2020; Braga et al., 2023; Kanberg et al., 2020; Virhammar et al., 2020; Edén et al., 2021; Khalil et al., 2018; Aamodt et al., 2021; De Lorenzo et al., 2021; Frontera et al., 2022; Marchegiani et al., 2023; Masvekar et al., 2022; Plantone et al., 2022; Prudencio et al., 2021; Sun et al., 2022) days vs. 20 (Aamodt et al., 2021; De Lorenzo et al., 2021; Frontera et al., 2022; Marchegiani et al., 2023; Masvekar et al., 2022; Plantone et al., 2022; Prudencio et al., 2021; Sun et al., 2022; Ramos-Casals et al., 2021; Ameres et al., 2020; de Boni et al., 2022; Hanson et al., 2022; Hay et al., 2021; Paterson et al., 2021 ; Sahin et al., 2023; Sun et al., 2021) days, p = 0.01).

Each patient in our study participated in a cognitive assessment at 6.5 ± 1.3 months after ICU discharge. Median MoCA score was 26 (Hay et al., 2021; Paterson et al., 2021 ; Sahin et al., 2023; Sun et al., 2021). Fig. 1 shows the results of the cognitive tests included in our test battery for all patients (n = 96), compared to data derived from a normal population. In total, 26 patients (27 %) performed in the impaired range (i.e., <1.5 SD from normative mean) on two or more cognitive tests and were classified as *cognitively impaired*. The incidence of cognitive impairment did not significantly differ between the three treatment groups (18 % (n = 5) for D-T-, 27 % (n = 7) for D + T-, and 33 % (n = 14) for D + T + patients; p = 0.36). Patients that did or did not develop cognitive impairments were similar in ICU-admission parameters, clinical course and delirium incidence. More detailed clinical information about this can be found in a previous publication (Duindam et al., 2022).

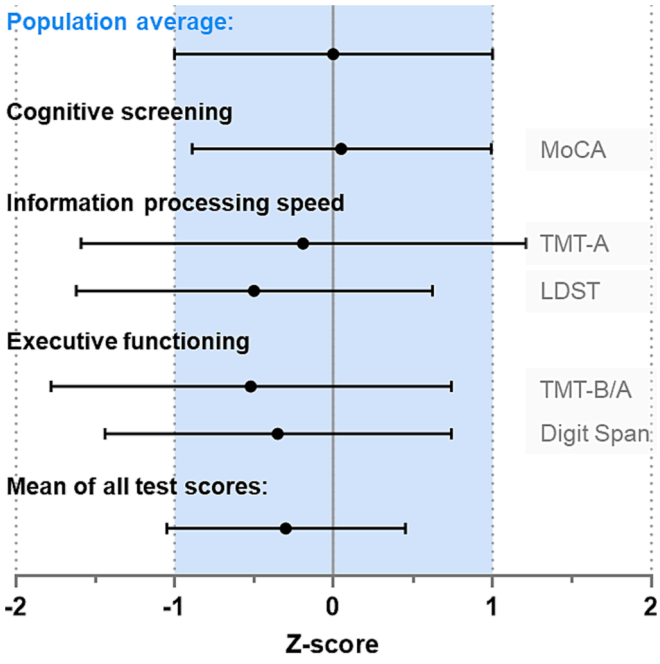

Fig. 1. Results of neuropsychological assessments six months after discharge from the Intensive Care unit (ICU). Values are presented in z-scores as mean ± SD. All test results are normalized for age, gender and education. **Abbreviations:** MoCA, Montreal Cognitive Assessment; TMT-A, Trail Making Test part A; LDST, Letter Digit Substitution Test; TMT-B/A, Trail Making Test interference B/A; Digit Span, Wechsler Adult Intelligence Scale-IV Digit Span test; Mean of all test scores, a composite score by calculating the mean z-score of all individual neuropsychological assessments.

3.2. Markers of systemic inflammation and neuroaxonal damage

We first assessed the effect of COVID-19 on systemic inflammation and neuroaxonal damage in solely the first cohort (D-T-), to avoid the potential effect from immunomodulatory medication. As depicted in Fig. 2, cytokine concentrations up to 28 days after ICU admission indicated considerable variation over time and among patients. Overall, the highest concentrations could be found on ICU admission, after which a gradual decline was observed. At the same time, NfL plasma concentrations showed a notable increase over time (Fig. 3 and Supplementary Table S1), from 24 pg/mL (Prudencio et al., 2021; Sun et al., 2022; Ramos-Casals et al., 2021; Ameres et al., 2020; de Boni et al., 2022; Hanson et al., 2022; Hay et al., 2021; Paterson et al., 2021 ; Sahin et al., 2023; Sun et al., 2021; Guasp et al., 2022; Kanberg et al., 2021; Needham et al., 2022; Peluso et al., 2022; Bonetto et al., 2022; Smeele et al., 2022; RIVM, 2022; Horby et al., 2021; Investigators et al., 2021; Levey et al., 2006; Nasreddine et al., 2005; Partington and Leiter, 1949) at ICU admission to 250 pg/mL [160–271] after four weeks (p < 0.001).

As a result of immunomodulatory therapy, circulating concentrations of cytokines were different compared to patients in the D-T- cohort (see Supplementary Fig. S1 and Table S2 for more detailed results). For instance, dexamethasone treatment resulted in significantly lower concentrations of IL-6, IL-1RA, and MCP-1 at ICU admission (Table S2). Additionally, the effect of treatment with tocilizumab was particularly noticeable for IL-6 concentrations; patients who received tocilizumab revealed significantly higher levels of IL-6 (68 [19–135] pg/mL) at ICU admission than those in the D-T- (33 [21–68] pg/mL) and D + T- (8 (Manca et al., 2021; Matschke et al., 2020; Schurink et al., 2020; Braga et al., 2023; Kanberg et al., 2020; Virhammar et al., 2020; Edén et al., 2021; Khalil et al., 2018; Aamodt et al., 2021; De Lorenzo et al., 2021; Frontera et al., 2022; Marchegiani et al., 2023; Masvekar et al., 2022; Plantone et al., 2022; Prudencio et al., 2021; Sun et al., 2022; Ramos-Casals et al., 2021; Ameres et al., 2020; de Boni et al., 2022; Hanson

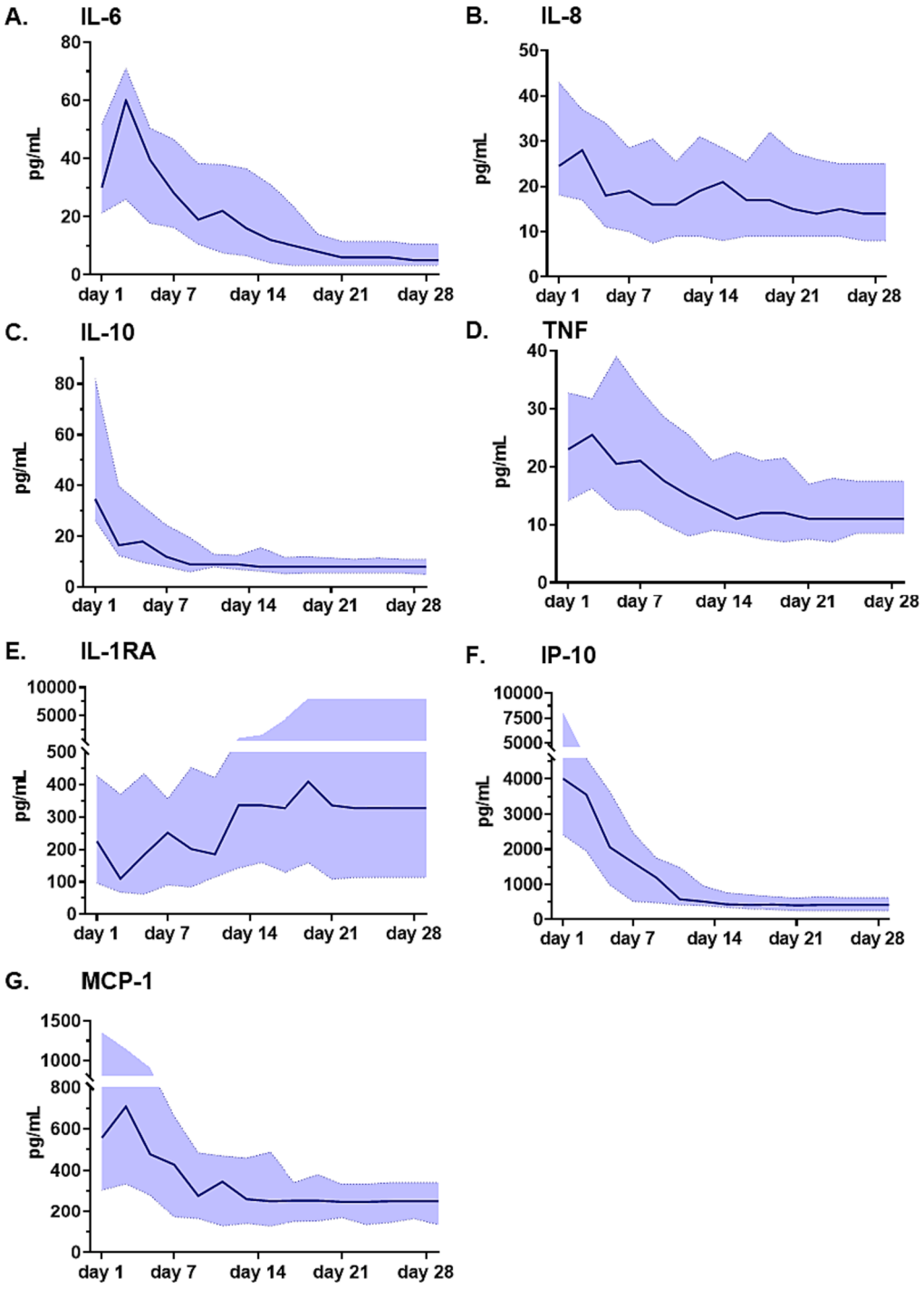

Fig. 2. Systemic inflammatory marker trajectory in immunomodulation-naive COVID-19 ICU patients during admission. The concentrations of the following plasma cytokines are presented: (A) Interleukin-6; (B) Interleukin-8; (C) Interleukin-10; (D) Tumor Necrosis Factor; (E) Interleukin-1 Receptor Antagonist; (F) Interferon-γ-induced Protein 10; (G) Monocyte chemoattractant Protein-1. Median + IQR of plasma levels in picogram per milliliter (pg/mL) are depicted. If a patient was discharged before 28 days, the last observation was carried forward in the graph. When assessing pair-wise differences (using Mann-Whitney U-tests) between the cytokines at day 14 with and without using the last-observation carried forward approach; no statistical significant differences were found: IL-6p = 0.23, IL-8p = 0.17, IL-10p = 0.21, TNF p = 0.14, IL-1RA p = 0.38, IP-10p = 0.63, MCP-1p = 0.50.

et al., 2022; Hay et al., 2021; Paterson et al., 2021 ; Sahin et al., 2023) pg/mL) treatment groups ($p < 0.001$). This effect persisted until day 14 and is a known phenomenon, due to blockage of IL-6 receptors, consequently allowing the accumulation of IL-6 in the circulation (Keske et al., 2020).

In the second cohort, NfL concentrations were solely measured on two time points during ICU stay (i.e. at ICU admission and after 14 days). NfL concentrations at ICU admission were slightly lower in D + T- and D + T + patients (18 (Khalil et al., 2018; Aamodt et al., 2021; De Lorenzo et al., 2021; Frontera et al., 2022; Marchegiani et al., 2023; Masvekar et al., 2022; Plantone et al., 2022; Prudencio et al., 2021; Sun et al., 2022; Ramos-Casals et al., 2021; Ameres et al., 2020; de Boni et al., 2022; Hanson et al., 2022; Hay et al., 2021; Paterson et al., 2021 ; Sahin et al., 2023; Sun et al., 2021; Guasp et al., 2022; Kanberg et al., 2021;

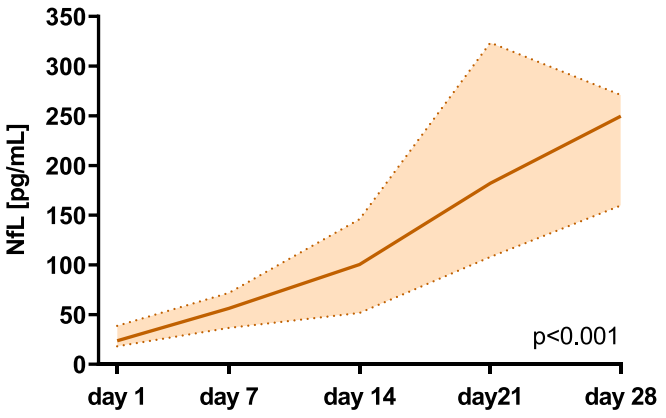

Fig. 3. Plasma concentrations of neurofilament light (NfL) in COVID-19 patients during admission to the Intensive Care Unit (ICU). Data are expressed as median + IQR in picograms per milliliter (pg/mL).

Needham et al., 2022; Peluso et al., 2022; Bonetto et al., 2022; Smeele et al., 2022) pg/mL and 17 (Khalil et al., 2018; Aamodt et al., 2021; De Lorenzo et al., 2021; Frontera et al., 2022; Marchegiani et al., 2023; Masvekar et al., 2022; Plantone et al., 2022; Prudencio et al., 2021; Sun et al., 2022; Ramos-Casals et al., 2021; Ameres et al., 2020; de Boni et al., 2022; Hanson et al., 2022; Hay et al., 2021; Paterson et al., 2021) pg/mL, respectively) compared to D-T- patients of the first cohort (24 (Prudencio et al., 2021; Sun et al., 2022; Ramos-Casals et al., 2021; Ameres et al., 2020; de Boni et al., 2022; Hanson et al., 2022; Hay et al., 2021; Paterson et al., 2021 ; Sahin et al., 2023; Sun et al., 2021; Guasp et al., 2022; Kanberg et al., 2021; Needham et al., 2022; Peluso et al., 2022; Bonetto et al., 2022; Smeele et al., 2022; RIVM, 2022; Horby et al., 2021; Investigators et al., 2021; Levey et al., 2006; Nasreddine et al., 2005; Partington and Leiter, 1949) pg/mL, p = 0.03). At day 14 of admission, a similar pattern was observed, with D + T- and D + T + patients exhibiting lower levels of NfL (54 [29–132] pg/mL and 59 [33–88] pg/mL, respectively) compared to D-T- patients (101 [52–146] pg/mL), (p = 0.06, Supplementary Table S1). During the course of ICU admission, 95 % of patients (n = 91) showed an increase of NfL (Supplementary Fig. S2).

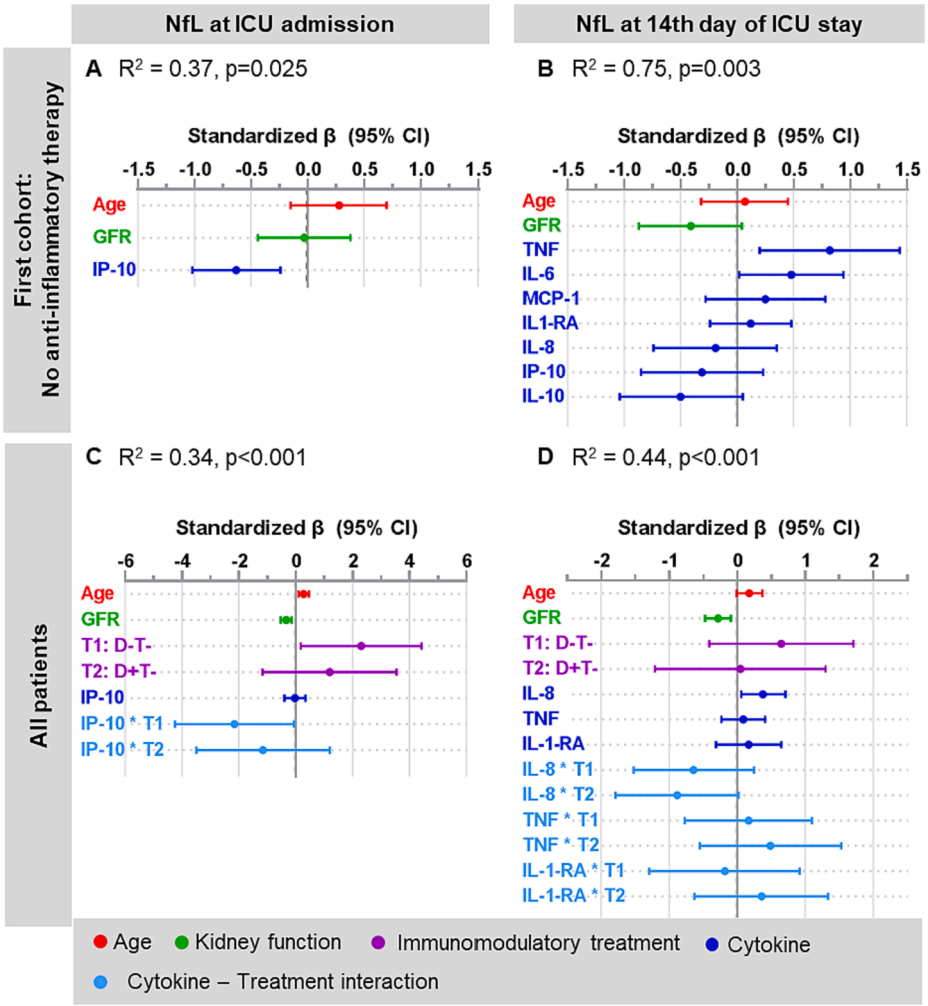

Fig. 4. Standardized predictor estimates for NfL based on multivariable regression models. An individual cytokine was inserted into the model if univariate correlations with NfL revealed a p-value < 0.1. Age, GFR were always included in the linear regression model. **A)** NfL at admission predicted by variables at ICU admission in patients without anti-inflammatory therapy. **B)** NfL on the 14th day of ICU admission predicted by variables at day 14 in patients without anti-inflammatory therapy. **C)** NfL at admission predicted by variables at ICU admission in all patients, regardless of anti-inflammatory therapy. **D)** NfL at 14th day of ICU admission predicted by variables at day 14 in all patients, regardless anti-inflammatory therapy. **Abbreviations:** ICU, Intensive Care Unit; GFR, Glomerular Filtration Rate; D-T-, no anti-inflammatory treatment; D + T-, dexamethasone as treatment; D + T+, dexamethasone and tocilizumab as treatment; IL, interleukin; TNF, Tumor Necrosis Factor; IL-1RA, Interleukin-1 Receptor Antagonist; IP-10, Interferon-γ-induced Protein 10; MCP-1, Monocyte Chemoattractant Protein-1; T1, treatment 1 (dexamethasone); T2, treatment 2 (dexamethasone and tocilizumab).

3.3. The correlation of systemic inflammation and neuroaxonal damage

Next, we assessed the association between plasma inflammation markers and neuroaxonal damage, first in the D-T- cohort. At the moment of ICU admission, univariate correlation analyses between all cytokines and NfL showed that only the anti-inflammatory IP-10 significantly (and inversely) correlated with NfL ($r = -0.54$, $p = 0.007$). In contrast, at day 14 all plasma cytokines, except for IL-1RA correlated significantly ($p < 0.05$) with NfL (more details are listed in Supplementary Table S3). Additionally, we used age, GFR and all cytokines that individually correlated with NfL with a p-value < 0.1, as independent predictors in a multiple linear regression analysis, where NfL was the dependent variable. Fig. 4A and 4B display the standardized predictor estimates for these models. The established prediction model for ICU admission ($R^2 = 0.37$, $F(3,20) = 3,87$, $p = 0.03$) showed similar results as the univariate analyses, as IP-10 appeared to be the only significant predictor, negatively correlating with NfL (standardized β −0.63, 95 % CI −1.02 to −0.24, $p = 0.003$). The prediction model for ICU day 14 was much stronger ($R^2 = 0.75$, $F(9,15) = 4.96$, $p = 0.003$), and showed significant positive correlation coefficients for TNF and IL-6.

Additionally, we assessed whether cytokine concentrations were correlated to NfL concentrations in all patients, with age, immunomodulatory treatment, GFR and cytokine × treatment interactions as additional independent variables. For ICU admission, the following covariates were added to the linear regression model with NfL as dependent variable: age, treatment and GFR at admission, and IP-10 and IP10 × treatment (the only cytokine showing an univariate correlation with a p-value < 0.1; results of univariate correlation analyses between cytokines and NfL can be found in Supplementary Table S3). This resulted in a model with an R^2 of 34 % ($F(7,84) = 6.22$, $p < 0.001$, Fig. 4C). At day 14 of ICU admission, IL-8, TNF and IL1-RA were added in the regression analysis to predict NfL This resulted in a model with an R^2 of 44 % ($F(13,79) = 4.82$, $p < 0.001$). Fig. 4D displays the standardized predictor estimates for this model. Of note, cytokines at admission (IL-8 and TNF) were able to significantly predict NfL levels at day 14, but this model performed less well compared to the model using cytokines of day 14 (R^2 of 31 %, $F(10,82) = 3.66$, $p < 0.001$).

Besides cytokines, C-reactive protein (CRP) can be used as a reliable and more generalized marker for (systemic) inflammation, especially in COVID-19 patients that did not receive immunomodulatory therapy. Interestingly, CRP at day 14 also showed strong correlations with NfL at day 14 (R^2 of 24 %, $F(1,26) = 8.50$, $p = 0.007$). Corroborating the findings of cytokines on day 14. This correlation still exists when using patients from both cohorts, although weaker (R^2 of 5 %, $F(1.94) = 4.88$, $p = 0.03$). This was not the case for ICU admission (D-T- cohort: R^2 of 0.0 %, $F(1,26) = 0.01$, $p = 0.94$; all patients: R^2 of 0.2 %, $F(1,93) = 0.27$, $p = 0.60$).

3.4. Long-term cognitive outcomes and their correlation with initial systemic inflammation and neuroaxonal damage markers

For both ICU admission and day 14 of ICU stay, there were no significant differences in C-reactive protein or individual cytokine concentrations between patients that showed cognitive impairment six months after discharge or those who did not (data on file). Furthermore, logistic regression between cytokines and cognitive outcomes (impaired/unimpaired) with age, immunomodulatory treatment, and GFR as covariates, did also not yield models that were significantly associated with cognitive outcomes (at admission: Nagelkerke $R^2 = 0.19$, $p = 0.56$; at ICU day 14: Nagelkerke $R^2 = 0.23$, $p = 0.55$), indicating that systemic inflammation and cognitive functioning could not be related in this cohort.

Next, we assessed whether plasma NfL concentrations were associated with long-term cognitive outcome. NfL levels at ICU admission revealed no statistical significant differences between patients that developed cognitive problems (median [IQR] 25.0 [16.0–37.9] pg/mL) compared to those who did not (17.3 [11.8–26.1] pg/mL, $p = 0.18$). However, patients with significantly elevated NfL at day 14 of their ICU stay, were more likely to develop cognitive impairment (median NfL of 82.7 [59.0–144.1] pg/mL for cognitively impaired patients vs 54.3 [32.2–107.0] pg/mL for non-impaired patients, $p = 0.005$).

Subsequently, to further assess whether the trajectory of NfL during ICU stay was correlated with long-term cognitive outcome, using linear mixed effect (LME) models, including correction for age and GFR, indeed showed that patients with a higher plasma NfL during ICU stay were more likely to develop long-term cognitive impairment (time × cognitive outcome $p = 0.03$), see Fig. 5.

Further exploration of correlations between NfL concentrations and individual cognitive tests are listed in Supplementary Table S4. In brief, NfL at ICU admission did not show correlations with any of the cognitive assessments, neither did the slope of NfL (i.e. mean change in pg/mL of NfL per admission day). However, at day 14 NfL correlated negatively with two cognitive tests that both represent assessments of the cognitive domain *information processing speed,* signifying that higher NfL concentrations at day 14 of ICU admission were associated with lower information processing speed at long-term follow-up. These tests included the Trail Making Test part A ($r = -0.26$, $p = 0.01$) and Letter Digit Substitution Test ($r = -0.24$, $p = 0.02$).

Fig. 6 provides an overview of the observed pathophysiological cascade.

4. Discussion

In this study we examined the relationships between systemic inflammation, neuroaxonal damage, and long-term cognitive outcomes in patients admitted to the ICU because of severe COVID-19-induced respiratory failure. Critically ill COVID-19 patients showed a pronounced inflammatory response related to a progressive increase in the levels of neuroaxonal damage marker NfL. Subsequently, we found that higher NfL concentrations during severe COVID-19 were predictive of long-term cognitive impairment six months after discharge. In summary, these findings suggest that systemic inflammation-induced neuroaxonal damage during ICU admission is associated with impaired long-term cognitive functioning.

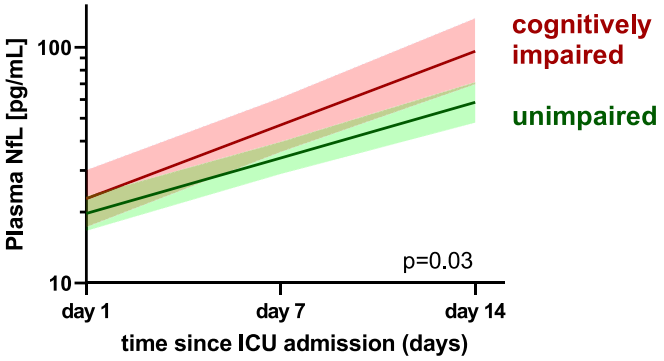

Fig. 5. Plasma NfL levels increase faster in COVID-19 patients with long-term cognitive impairment. NfL was measured in plasma samples collected on day 1, and day 14 after admission to ICU in 26 and 70 COVID-19 patients with or without cognitive impairment, respectively. Estimated trajectories for non-MCI (green) and MCI patients (blue) are drawn using mixed-effects modelling with an interaction term for time point and cognitive status. Age and GFR were inserted as covariates. Shaded areas indicate 95 % confidence intervals. NfL levels rise with time since admission to the hospital in all patients, but the slope of change is steeper in cognitively impaired subjects ($p = 0.03$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

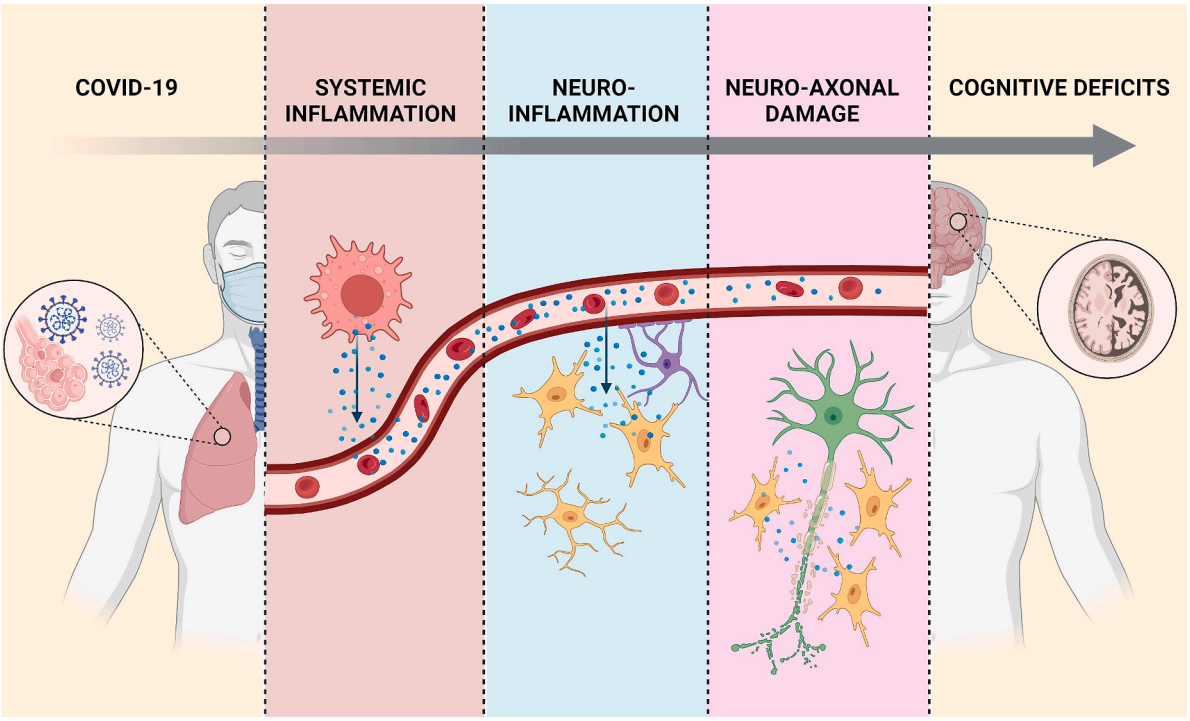

Fig. 6. Schematic representation of severe COVID-19 leading to neuropsychological problems through inflammation-induced brain injury. Tissue damage resulting from COVID-19 pneumonia induces a pronounced systemic inflammatory response, which can be measured by changes in circulating blood parameters, like leukocytes, CRP and pro- and anti-inflammatory cytokines. This inflammation subsequently activates the immune cells within the brain, specifically microglia and astrocytes, creating a neuroinflammatory environment within the CNS. Such neuroinflammation creates a detrimental microenvironment for neurons, causing neuronal dysfunction and apoptosis, measurable via biomarkers like neurofilament light (NfL). Ultimately, this cascade could potentially lead to clinical deterioration of the patient, evidenced by long-term cognitive decline, as can be assessed using neuropsychological assessments. **Abbreviations:** CRP, C-reactive protein; CNS: Central Nervous System; NfL: neurofilament light chain. *Created with BioRender.com.*

4.1. Neurofilament light in the context of systemic inflammation

Our results showed that at ICU admission only IP-10 correlated with NfL levels. However, at day 14, higher levels of CRP and several cytokines correlated with increased NfL levels, indicating the presence of neurotoxic effects of an inflammatory load due to *prolonged* systemic inflammation. Consecutively, these higher NfL levels were associated with more impaired cognition at long-term follow-up, while the direct association between inflammatory cytokines and impaired long-term cognitive functioning was far less pronounced. As such, our results suggest that sustained systemic inflammation might be associated with more neuroaxonal damage, which might in turn be associated with stronger and clinically significant post-COVID-19 cognitive decline. While we did not directly measure neuroinflammation in this study, as has been conducted by a cross-sectional study on long-COVID (Braga et al., 2023), we hypothesize that systemic inflammation impacts the brain through the activation of neuroinflammatory pathways (Peters van Ton et al., 2021; Cunningham et al., 2009; DiSabato et al., 2016), leading to a disrupted homeostasis and neuroaxonal damage. The importance of systemic inflammation in the development of neuroaxonal damage is also supported by a study showing that NfL was significantly lower in patients with hospitalized interstitial pulmonary fibrosis (who showed oxygenation problems, but had no acute systemic inflammation) compared to hospitalized COVID-19 patients (who showed oxygenation problems and also had pronounced acute systemic inflammation) (Plantone et al., 2022). Future mechanistic studies in COVID-19 models are required to corroborate our hypothesis, in particular for the causality of the respective mechanisms.

4.2. Neurofilament light in the context of development of cognitive function

The occurrence of prolonged symptoms after COVID-19 (long-COVID, often fatigue, malaise or cognitive dysfunction) has been reported in large numbers of patients (Taquet et al., 2021; Peter et al., 2022; Michelen et al., 2021). In accordance, we found that up to a third of our cohort showed cognitive impairment at six months post-hospital discharge (Duindam et al., 2022). Previous studies measuring NfL in COVID-19 patients lacked extensive phenotyping of patients regarding inflammation and NfL trajectories over both short-term as well as long-term timeframes, along with detailed neuropsychological assessments. As such, important knowledge gaps existed: NfL in relation to cognitive decline and systemic inflammation as a potential mechanism underlying the development of persistent complaints after severe COVID-19 (referred to as 'long-COVID'). Our study used extensive neuropsychological tests to objectively determine cognitive dysfunction six months after ICU discharge. More detailed outcomes of these assessments have been published previously (Duindam et al., 2022). Notably, the results of the current study show that elevated plasma NfL levels are correlated with impaired performance on two *information processing speed* assessments, namely TMT-A and LDST. This finding is in line with the results in non-COVID-19 disorders showing that axonal damage in the brain (i.e., white matter damage) impairs information processing speed. For instance, patients with Multiple Sclerosis (MS), a progressive demyelinating disease causing axonal damage, often exhibit cognitive dysfunction (Chiaravalloti and DeLuca, 2008; Ruano et al., 2017). NfL has been identified as a biomarker for disease progression in MS (Kuhle et al., 2019), but it has also been found to be linked to specific impairments in the domain of information processing speed (Gaetani et al., 2019). Furthermore, previous studies have shown positive correlations

between elevated plasma NfL levels and cognitive performance in traumatic brain injury, e.g. in boxers (Neselius et al., 2014).

4.3. Neurofilament light in the context of (critical) illness

Age is strongly linked to increased levels of NfL (Khalil et al., 2020). Recent research has also identified kidney function as a critical predictor of NfL in hospitalized patients (Fitzgerald et al., 2022). Our findings also indicate that glomerular filtration rate (GFR) explains at least some of the variation in NfL levels in ICU patients. In patients with profound systemic inflammation, such as those with sepsis or COVID-19, renal clearance may be either impaired, but can also be augmented (Beunders et al., 2021). This can result in relatively increased or decreased NfL levels in plasma, respectively, and necessitates GFR correction to accurately interpret NfL findings in research or clinical settings.

In hospitalized patients, NfL concentrations are usually measured within the first 48 h of admission, neglecting their trajectory during the course of the illness (Kanberg et al., 2020; Virhammar et al., 2020; Aamodt et al., 2021; Frontera et al., 2022; Marchegiani et al., 2023; Prudencio et al., 2021; Ameres et al., 2020; de Boni et al., 2022; Hanson et al., 2022; Hay et al., 2021; Paterson et al., 2021 ; Sahin et al., 2023; Sun et al., 2021; Sutter et al., 2021; De Lorenzo et al., 2021; Fisse et al., 2021). The COVID-19 pandemic provided an opportunity to assess the trajectory of NfL levels in a uniform group of critically ill ICU patients (Kanberg et al., 2020; Smeele et al., 2022; Fisse et al., 2021), confirming a gradual increase during ICU stay. The results of our study show that NfL levels after two weeks of ICU stay are more indicative of long-term cognitive outcomes, than a sample drawn at admission. In line with this, a study of critically ill patients with mainly bacterial sepsis showed that NfL levels increased significantly during the first week (Ehler et al., 2019). This highlights the importance of obtaining NfL levels later on during ICU stay, as they are likely to better correspond to the cumulative load of neuronal damage acquired during a critical illness episode.

4.4. Strengths and limitations

Our present study combines systemic inflammation and NfL trajectories and relate this to long-term cognitive outcomes, offering valuable new insights. Despite the limited size of our cohort from a single center, we conducted detailed phenotyping that included serial sampling up to 28 days allowing assessment of biomarker dynamics during severe COVID-19, analysis of the interaction between systemic inflammation and neuroaxonal damage over time, and quantification of long-term cognitive functioning. This enabled us to demonstrate the relationships between sustained systemic inflammation, elevated increased axonal damage - as indicated by increased NfL plasma concentrations – and long-term cognitive dysfunction. We were able to study systemic inflammation and NfL concentrations both in first surge patients who did not receive any immunomodulatory therapy, as well as in those treated with dexamethasone and tocilizumab. This facilitated an investigation into the effects of COVID-19 itself on inflammation and neuroaxonal damage, as well as the potential therapeutic effects of the immunomodulatory treatments that have become standard care for COVID-19. However, as a consequence of the limited size of our cohort, statistical power was insufficient to come to conclusions from further subgroup analyses focusing on patients who received immunomodulatory therapy. Our study has additional limitations. First, the exclusion of patients who declined to participate in long-term cognitive assessments, as well as those who did not survive until 6 months after hospital discharge may have caused an underestimation of NfL levels and an overestimation of cognitive functioning. Second, inherent to this group of patients, we lacked pre-morbid information on NfL concentrations and cognitive functioning. Nonetheless, we argue that this is not a major issue, given that NfL concentrations at ICU admission displayed minimal variation within the cohorts and our finding that concentrations measured at day 14 demonstrated a better correlation with cognitive dysfunction.

5. Conclusion

In patients with severe COVID-19 who are admitted to the ICU, sustained systemic inflammation was associated with higher NfL concentrations over time, indicative of neuroaxonal damage. NfL concentrations progressively increased during ICU stay and predicted future long-term cognitive decline.

CRediT authorship contribution statement

H.B. Duindam: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration. **D. Mengel:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Visualization. **M. Kox:** Methodology, Supervision, Writing – review & editing. **J.C. Göpfert:** Methodology, Resources, Investigation, Writing – review & editing. **R.P. C. Kessels:** Methodology, Supervision, Writing – review & editing. **M. Synofzik:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Funding acquisition. **P. Pickkers:** Conceptualization, Methodology, Supervision, Writing – review & editing, Funding acquisition. **W.F. Abdo:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT, OpenAI in order to improve readability and language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2024.02.002.

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