Supplementary materials

Supplementary Methods

We adopted the following search terms: ((neurofilament light chain[Title/Abstract]) OR (NFL[Title/Abstract])) AND ((intensive care[Title/Abstract]) OR (cardiac arrest[Title/Abstract]) OR (critical illness[Title/Abstract]) OR (perioperative care[Title/Abstract]) OR (surgery[Title/Abstract]) OR (sepsis[Title/Abstract]) OR (delirium[Title/Abstract]) OR (atrial fibrillation[Title/Abstract]) OR (cardiovascular risk factors[Title/Abstract]) OR (diabetes[Title/Abstract]) OR (hypertension[Title/Abstract]) OR (COVID-19[Title/Abstract]) OR (SARS-CoV-2[Title/Abstract]) OR (HIV[Title/Abstract]) OR (human immunodeficiency virus[Title/Abstract]) OR (AIDS[Title/Abstract]) OR (acquired immunodeficiency syndrome[Title/Abstract]) OR (schizophrenia[Title/Abstract]) OR (bipolar disorder[Title/Abstract]) OR (anorexia[Title/Abstract]) OR (major depressive disorder[Title/Abstract]) OR (depression[Title/Abstract]) OR (aging[Title/Abstract]) OR (preeclampsia[Title/Abstract]) OR (diving[Title/Abstract]) OR (hyperbaric[Title/Abstract]) OR (acute mountain sickness[Title/Abstract]) OR (psoriasis[Title/Abstract])).
Supplementary Figure 1. Flow-chart for the selection of studies

Identification of studies via databases and registers

Records identified from database (PubMed) searching (n=615) → Records removed before screening: duplicates (n=8)

Records screened (n=607)

Full-text articles assessed for eligibility (total n=607) → Articles excluded because not pertinent or not relevant to the purpose of the review (n=493)

Studies included in the review (n=114)
  • Intensive care (n=18)
  • Perioperative care (n=16)
  • Atrial fibrillation and cardiovascular risk factors (n=14)
  • Infectious diseases (n=25)
  • Psychiatric disorders (n=18)
  • Normal aging (n=12)
  • Other conditions (n=11)
Supplementary Table 1. Main studies exploring NfL performance in the adult and pediatric ICU setting and mostly in patients after cardiac arrest.

| Paper                          | Country of studied population | Examined biofluid | Assay | N    | Primary outcome and timing                                      | Sample timing (after CA) | Best AUC (sample timing) | Sens (%) | Spec (%) | Cut-off | Other biomarker performance | Other significant findings |
|--------------------------------|-------------------------------|-------------------|-------|------|---------------------------------------------------------------|--------------------------|--------------------------|----------|----------|---------|------------------------------|----------------------------|
| Andersson et al. 2021          | Multicentric: Europe and Australia | Serum            | Simoa | 939  | Adults with out of hospital cardiac arrest                    | 24, 48, 72 hours          | 0.92 when NfL analyzed alone, Approx. 0.95-0.96 in models including NfL, other research-grade biomarkers, clinical variables and clinically accessible biomarkers (Days 1-3) | -        | -        | -       | NSE, S100B, TrtT, BNP, and PCT. AUC 0.94 in model including also clinical variables (Day 3) | The models which included NSE after 72 h and NfL on any of the three days showed promising prognostic performance |
| Disanto et al. 2019            | Switzerland                   | Serum             | Simoa | 14  | Adults (one with 2 cardiac arrest)                            | Median 2 (1-3) days, range (0-17 days) | 0.98                     | 83       | 100      | 3437 pg/ml | NSE; AUC 0.80                | Positive associations between serum NfL and time to return of spontaneous circulation, NSE concentration and severity of brain damage on EEG. High NfL levels in samples collected up to 17 days after cardiac arrest |
| Fisse et al. 2020              | Germany                       | Serum             | Simoa | 35  | Adults admitted to ICU (45% with resuscitation)               | Day 1 and every 7 days during ICU treatment | -                       | -        | -        | -       | -                            | Elevated NfL in all ICU patients. Maximum of NfL levels at day 35 of ICU treatment. mRS at the end of follow-up correlated with NfL level at admission. No significant differences in NfL levels between patients with and without critical illness polyneuropathy/neuromyopathy at any timepoint |
| Goeral et al. 2020             | Austria                       | Serum             | Simoa | 48  | Preterm infants with PIVH                                    | From 3 to 7 time points (not specified time) | 0.71                     | -        | -        | -       | -                            | -                          |

CA: Cardiac arrest; CPC: Clinical Predictive of Cerebral Performance; CA: Cardiac arrest; N: Number; NfL: Neurofilament light; NSE: Neuron-specific enolase; S100B: S100 calcium-binding protein B; TrtT: Troponin T; BNP: B-type natriuretic peptide; PCT: Procalcitonin; mRS: Modified Rankin Scale; PIVH: Porencephalic Intraventricular Hemorrhage; Sens: Sensitivity; Spec: Specificity; Cut-off: Cut-off value; AUC: Area under the curve;pg/ml: picogram per milliliter.
| Study                  | Country                        | Sample Type | Sample Size | Sample Description | Test | Outcome | Time点 | Sensitivity | Specificity | AUC | Concentration | Conclusion |
|------------------------|--------------------------------|-------------|-------------|--------------------|------|---------|-------|-------------|-------------|-----|---------------|------------|
| Hunziker et al. 2021   | Switzerland                    | Serum       | 164        | adults with out of hospital cardiac arrest | CPC   | Day 1   | 66/98 | 0.77        | 72          | 82  | 50 pg/ml      | Adding NfL to the clinical risk score for out of hospital cardiac arrest and CAHP significantly improved the discriminative power between good and poor neurological outcome |
| Kirschen et al. 2020   | USA                            | Serum       | 32         | children with ARDS and out of hospital cardiac arrest; 18 healthy controls | Survival | Within 24 hours (from ARDS onset) | 13/19 | 0.78        | -           | -   | -             | -          |
| Moseby-Knappe et al. 2019 | Multicentric: Europe and Australia | Serum      | 717        | adults with out of hospital cardiac arrest | CPC at 6 months | 357/360 | 24, 48, 72 hours | 0.94 (Day 3) | 70          | 99  | 1122 pg/ml   | At comparable specificities, serum NfL showed greater sensitivity for poor outcome compared to EEG, SSEP, CT and clinical tests |
| Rana et al. 2013       | Germany                        | ELISA       | 85         | adults with out of hospital cardiac arrest | mGOS at 6 months | 43/18  | Days 1 (within 2 hours), 2, 3, 5, 7 | 0.99 (Day 7) | 94          | 100 | 252 pg/ml    | -          |
| Rosén et al. 2004      | Sweden                         | ELISA       | 22         | adults with out of hospital cardiac arrest | GOS at best-recorded result at any of the time points (12 days -1 year) | 10/12 | Mean 17.5, range 12-30 days | -            | 92          | 90  | 9622 mg/L    | NIL levels correlated well with anoxia time and coma depth |
| Rosén et al. 2014      | Sweden                         | ELISA       | 21         | adults with out of hospital cardiac arrest | GOS at best-recorded result at any of the time points (12 days -1 year) | 10/11 | 12-14 days | -            | -           | -   | -             | NIL, t-tau and YKL-40, but not Aβ38, Aβ40, Aβ42 were significantly altered after cardiac arrest; biomarker changes were more pronounced in cases with poor outcome |
| Shah et al. 2018       | UK                             | Plasma      | 26         | newborns with hypoxic-ischemic encephalopathy treated with therapeutic hypothermia for 72 h | MRI pattern predictive of outcome | 13/13 | At target temperature, prior to rewarming and after rewarming | 0.97 (after rewarming) | 92          | 92  | 417 pg/ml    | -          |
| Wilshessari et al. 2021 | Finland and Denmark            | Plasma      | 112        | adults with out of hospital cardiac arrest | CPC at 6 months | 73/39  | Admissions n, 24, 48, 72 hours | 0.98 (Day 3) | 85          | 99  | 344 pg/ml    | Higher mean arterial pressure was associated with lower NfL concentrations |
| Protein/Marker | Sensitivity | Specificity | AUC 0.70 (Day 3) |
|---------------|-------------|-------------|------------------|
| S100B         |              |             | Hospital cardiac arrest |

Aβ38, 40, 42: amyloid beta peptide 1-38, 1-40, 1-42; AUC: area under the curve; BNP: Brain Natriuretic Peptide; CAHP: cardiac arrest hospital prognosis score; CPC: cerebral performance categories scale; CSF: cerebrospinal fluid; CT: computed tomography; GOS: Glasgow Outcome scale; ICU: intensive care unit; mGOS: modified Glasgow Outcome Score; mRS: modified Rankin Scale; NSE: neuron-specific enolase; ELISA: Enzyme-Linked Immunosorbent Assay; GOS: Glasgow outcome score; ICU: intensive care unit; NfL: neurofilament light chain protein; PCT: procalcitonin; PIVH: peri/intraventricular hemorrhage; Sens: sensitivity; Spec: specificity; S100: S100 calcium-binding protein B; SSEP: somatosensory evoked potentials; TnT: troponin; t-tau: total tau protein; YKL-40: Chitinase 3-like 1; -: not reported
## Supplementary table 2. Main studies exploring NfL performance in COVID-19

| Paper | Country of studied population | Examined biofluid | Assay | N | Study design | NfL performance | Other biomarker performance |
|-------|------------------------------|------------------|-------|---|--------------|-----------------|-----------------------------|
| Aamodt et al. 2021<sup>15</sup> | Norway and Sweden | Serum | Simoa | 47 | Cross-sectional and longitudinal | Higher blood NfL levels in COVID-19 non-survivors upon admission (p<0.001) than patients who were discharged alive both in adjusted analyses (p=2.6x10⁻⁷) and unadjusted analyses (p=0.001). The concentrations of NfL in non-survivors increased over repeated measurements, whereas the concentrations in survivors were stable. | Higher GFAP in non-survivors than survivors (p=0.02). |
| Ameres et al. 2020<sup>16</sup> | Germany | Serum | Simoa | 100 | Cross-sectional | Higher serum NfL levels in patients with COVID-19 compared to controls stratified for age group. COVID-19 status was significantly associated with serum NfL levels in a multivariable linear regression model including COVID-19 status, age and sex as independent variables (b=1.87; p=0.05) | - |
| Bozzetti et al. 2021<sup>17</sup> | Italy | Serum | Simoa | 107 | Prospective | Among patients with available onset and 6-month follow-up samples (n=29), 14 cases had increased NfL levels in the acute stage in comparison with healthy controls. A significant decrease (p<0.001) of NfL values was observed over time in patients with available onset and follow-up sera. No significant difference in serum NfL levels between patients with vs without persistent neurological symptoms at 6-month follow-up. | - |
| de Lorenzo et al. 2021<sup>18</sup> | Italy | Serum | Simoa | 104 | Cross-sectional and prospective | Higher serum NfL levels, as measured the day of the emergency room admission, in patients with fatal outcome and in those needing ICU transfer. | Higher serum UCH-L1, GFAP and tau concentrations, as measured the day of the emergency room admission, in patients with fatal outcome and higher UCH-L1 concentration in those needing ICU transfer. Serum tau levels at the admission predicted mortality with the best AUC (0.7622) |
| Study | Country | Fluid | Method | Patients with COVID-19 and neurological symptoms | Case Series | Findings |
|-------|---------|-------|--------|--------------------------------------------------|-------------|----------|
| Edén et al. 2020<sup>13</sup> | Sweden | CSF | ELISA | 6 patients with moderate to severe COVID-19 and neurological symptoms (encephalopathy 4/6, meningitis 1/6, dysgeusia 1/6) | Case series (cross-sectional) | Higher CSF NfL levels in two patients with COVID-19-related encephalopathy. The median (range) age-adjusted CSF NfL (65) was 974 (669-1998) ng/L with an upper normal reference value of 1577 ng/L. Increased CSF and serum neopterin concentration in all patients with median (range) neopterin concentrations of 43.0 (26.7-50.0) in CSF and 41.9 (38.6-44.4) nmol/L in serum and upper normal reference values of 5.8 (CSF) and 8.8 (serum) nmol/l. |
| Espíndola et al. 2020<sup>14</sup> | Brazil | CSF | ELISA (Human Neurofilament ELISA kit, FineTest, China) | 58 patients with COVID-19 related neurological manifestations: headache (n=14); encephalopathy (n=24); inflammatory neurological diseases: meningocnecephalitis (n=4), acute myelitis (n=3), meningitis (n=2), acute disseminated encephalomyelitis (n=2), encephalitis (n=2), and neuromyelitis optica (n=1); and Guillain-Barré syndrome (n=6). | Cross-sectional | No significant difference in NfL levels between different groups. Patients with inflammatory neurological disease showed the highest median (IQR) CSF NfL levels: 3068 pg/mL (1410-6846). Among patients with inflammatory neurological disease, higher NfL levels in the CSF were associated with higher intracranial pressure ($r=0.817$; $p=0.002$) and total protein ($r=0.581$; $p=0.030$). CSF NfL levels were significantly higher in the critical COVID-19 group compared to the other severity categories. No significant difference in t-tau protein levels between different groups. |
| Frithiof 2021<sup>15</sup> | Sweden | Plasma | Simoa | 111 COVID-19 patients: 14 patients developed ICU-acquired weakness, out of which 11 patients were diagnosed with critical illness neuropathy/myopathy; 10 non-COVID-19 ICU control patients | Prospective | Analysis of biomarkers at early (median 4, IQR 3–9 days) and late (median 16, IQR 11–42 days) stages. Higher NfL levels in the critical illness neuropathy/myopathy group both at the early and late time points ($p=0.001$ and $p=0.03$, respectively) compared to cases without. Higher NfL concentration correlated with longer ICU time both for the group with critical illness neuropathy/myopathy (group 3, $p=0.02$; $r=0.4$) and without (group 4, $p=0.005$; $r=0.62$). For NfL, a trend towards significant correlation with CMAP ($r=-0.511$; $p=0.078$) and a correlation with fibular nerve motor amplitude ($r=-0.64$; $p=0.022$). Higher GFAP levels in the critical illness neuropathy/myopathy group at both early ($p=0.04$) and late ($p=0.02$) time points. Tau significantly increased in the critical illness neuropathy/myopathy cohort compared to patients without ($p=0.04$). For GFAP significant correlation with CMAP ($r=-0.72$; $p=0.007$). For tau, significant correlation with sural nerve sensory amplitude ($r=-0.59$, $p=0.036$) and CSA ($r=-0.63$; $p=0.024$). |
| Study                  | Country           | Sample Type | Biomarker | Overview                                                                 |
|-----------------------|-------------------|-------------|-----------|---------------------------------------------------------------------------|
| Geis et al. 2021**    | Germany, Switzerland | Serum       | SiMoa     | 2652 children, out of which 148 SARS-CoV-2 antibody positive with asymptomatic to moderate COVID-19 infection ** | No serum NfL difference between children with SARS-CoV-2 antibodies and those without. Multivariate regression analysis revealed neither antibody status, antibody levels, nor clinical severity as independent predictors of serum NfL. Follow-up of children with pediatric MIS showed no association with serum NfL. |
| Garcia et al. 2021*   | USA               | CSF         | SiMoa     | 18 COVID-19 patients with neurological complications categorized by diagnosis (stroke, encephalopathy, headache) and illness severity (critical, severe, moderate, mild). 82 controls (healthy, infectious and neuroinflammatory disorders and stroke controls).* | Higher CSF NfL levels in the COVID-19 stroke group as compared with healthy controls (p<0.001) and the COVID-19 headache group (p<0.01). Higher CSF NfL levels in patients with central neurological symptoms (GCS ≤ 12 or central weakness, n=10; 2930 ng/L [IQR=1810–12318]) compared to patients with other neurological symptoms (950 ng/L [IQR=405–1785], p<0.05). Higher CSF NfL levels in patients with severe or critical COVID-19 (2610 ng/L [IQR=1,280–4,705]) compared to mild and moderate disease (420 ng/L [IQR=385–1,640, p<0.05]). NfL correlated with COVID-19 severity grade (r=0.56, p<0.05) and number of days in the ICU (r=0.72, p<0.001) |
| Kanberg et al. 2020** | Sweden            | Serum       | SiMoa     | 47 patients with mild (n=20), moderate (n=9) or severe (n=18) COVID-19; 33 controls. ** | Higher plasma NfL levels in patients with severe COVID-19 compared to controls (p=0.001). Patients with severe COVID-19 had 208% (95% CI 120–329%) higher plasma NfL than controls. Higher plasma NfL concentrations in severe COVID-19 patients from a median (IQR) 20 (11–24) pg/mL at the initial to 32 (16–60) pg/mL at the last sampling (P=0.002) |

**Cross-sectional

* Higher CSF pro-inflammatory cytokines (IL-6, TNFa, IL-12p70) and IL-10 in CSF of COVID-19 and non-COVID-19 stroke subjects (similar) compared to controls. Worst GCS score correlated with CSF GFAP levels (r=-0.50, p<0.05).

**Cross-sectional

Higher plasma GFAP levels in patients with severe COVID-19 compared to controls (p=0.001). Patients with severe COVID-19 had 78% (95% CI 27-150%) higher plasma GFAP than controls. Decreased plasma GFAP concentrations in severe COVID-19 patients from a median (IQR) 215 (106–281) pg/mL at the initial to 103 (60-225) pg/mL at the last sampling (p=0.004).
| Study | Location | Type | Serum Component | Sample Size | Study Design | Key Findings |
|-------|----------|------|-----------------|-------------|--------------|--------------|
| Kanberg et al. 2021 | Sweden | Serum | Simoa NfL and GFAP, Elecsys electrochemiluminescence immunoassay for GDF-15 (Cobas platform; Roche Diagnostics, Rotkreuz, Switzerland). | 100 patients with mild (n=24), moderate (n=28), and severe (n=48) COVID-19 * | Prospective | Higher acute phase serum NfL levels in severe COVID-19 patients than all other groups (all p<0.001). After 6 months, NfL concentration had normalized. Correlation between acute phase serum NfL and GDF-15 concentration (r=0.53; p<0.001). No correlation between serum NfL levels and persistent neurological symptoms (fatigue - n=40, “brain-fog” - n=29, and changes in cognition - n=25) during the available clinical follow-up (median 225 days, IQR 187–262). |
| Mantovani et al. 2021 | Italy | Serum | Simoa | 37 COVID-19 survivors (testing positive in February-May 2020): 10 patients met the criteria for myalgic encephalomyelitis/chronic fatigue syndrome * | Cross-sectional | Serum NfL levels were normal in all patients, except one and did not differ comparing encephalomyelitis/chronic fatigue syndrome positive and negative groups. |
| Mariotto et al. 2020 | Italy | Serum | Simoa | 107 patients with COVID-19 without neurological comorbidities | Cross-sectional | Higher plasma NfL levels in COVID-19 patients admitted to the ICU and who underwent otracheal intubation as compared to COVID-19 patients admitted to the medical unit. |
| Masvekar et al. 2022 | Italy, USA | Serum | Simoa | 378 patients with COVID-19 and 138 controls (58 healthy controls, 10 COVID-19 negative controls, 35 multiple sclerosis non-active and 35 multiple sclerosis active controls) ** | Cross-sectional and prospective | Plasma NfL levels in COVID-19 patients were higher compared to healthy controls and increased with disease severity, but only cohorts of critically ill COVID-19 and multiple sclerosis patients reached statistical significance compared to healthy controls. Significant increase in NfL levels only in COVID-19 patients approaching to death when measured longitudinally at 5- to 10-day intervals from hospitalization. Plasma NfL levels remained elevated for weeks (up to 3 months) in patients who eventually survived. |
| | | | | | | Decreased ALC and increased LDH levels correlated with COVID-19 severity and significantly differed from healthy controls only in critically ill COVID-19 patients. ALC and LDH levels exhibited earlier variations and larger day-to-day fluctuations as compared to plasma NfL levels. |
| Study          | Country | CSF Source | Biomarker Assays | Sample Characteristics | Study Design | Findings                                                                 | Subgroup Findings |
|---------------|---------|------------|------------------|-------------------------|--------------|--------------------------------------------------------------------------|--------------------|
| Ngo et al. 2021 | USA     | CSF        | NfL MSD U-PLEX kit | 5 pediatric COVID-19 patients and 5 pediatric healthy controls. 3/5 COVID-19 patients met the case definition for multisystem inflammatory syndrome in children, whereas one patient had acute COVID-19 and febrile seizures with encephalopathy | Case series     | No difference in CSF NfL levels between COVID-19 and controls. 2/5 COVID-19 patients had highly elevated levels of NfL |                    |
| Paterson et al. 2021 | United Kingdom | CSF and serum | Simoa for serum NfL and GFAP; in-house ELISA for CSF NfL and GFAP; Lumipulse assays (Fujirebio, Ghent, Belgium) for t-tau, p-tau181, Aβ40 and Aβ42 | 152 individuals subdivided into 3 subgroups: 51 non-neurological COVID-19 patients, 43 non hospitalized, non-neurological COVID-19 patients, 34 neurological COVID-19 patients and 24 non-COVID controls | Cross-sectional | Higher CSF NfL levels in COVID-19 patients with inflammatory neurological complications (encephalitis and acute disseminated encephalomyelitis) compared to those with encephalopathy, Guillain-Barré syndrome and non-COVID controls (all p<0.0001). | Higher CSF t-tau in neurological CNS COVID-19 cases compared to PNS cases and non-COVID controls. Five individuals in the COVID-neurological group had increased CSF Aβ42/Aβ40 ratio (3 Guillain-Barré syndrome and 2 encephalopathy). Normal CSF p-tau181, CSF and blood GFAP concentrations in any group. |
| Pilotto et al. 2020 | Italy    | CSF        | In-house ELISA   | A 60-years-old patient with COVID-19 related encephalopathy | Case report   | Normal CSF NfL levels 8 days after symptoms onset (one day after starting high-dose intravenous steroid) | Normal t-tau levels. Slightly increased IL-6 levels (2.36 pg/mL), strongly increased IL-8 levels (higher than 1100 pg/mL), and increased TNF-alfa (1.31 pg/mL) and β2M (3.06 mg/L) levels. |                    |
| Pilotto et al. 2021 | Italy    | CSF        | In-house ELISA   | 13 patients with COVID-19 related encephalitis. 18 neurologically healthy controls (normal MRI, neurological examination and CSF analyses). 21 patients with other types of encephalitis (10 infectious, 11 autoimmune) ** | Cross-sectional | Higher CSF NfL levels in patients with COVID-19 related encephalitis as compared to healthy controls (p<0.001). Comparable CSF NfL levels in patients with COVID-19 related encephalitis as compared to encephalitis not associated with SARS-CoV-2 infection (p=0.026). | Higher CSF GFAP, t-tau, TREM2, β2M, YKL-40, IL-1β, IL-6, IL-8, TNF-α levels in patients with COVID-19 related encephalitis as compared to healthy controls (p<0.001). Comparable CSF levels of GFAP, t-tau and neuroinflammatory biomarkers in patients with COVID-19 related encephalitis as compared to encephalitis not associated with SARS-CoV-2 infection (p=0.026). |
| Study Reference | Country | Sample Type | Sample Description | Study Design | Findings |
|-----------------|---------|-------------|--------------------|--------------|----------|
| Prudencio et al. 2021 | USA | Serum | Serum Simoa | Cross-sectional and prospective | Higher serum NfL levels in COVID-19 patients compared to healthy controls \( (p<0.001) \). About 34% of COVID-19 patients had serum NfL concentration above a prespecified cut-off of at least 3 SDs the mean of healthy controls. Serum NfL concentrations were significantly higher in patients who needed mechanical ventilation, who were admitted to the ICU, who had a longer length of hospital stay, and who had a higher mRS at discharge \( (all \ p<0.001) \). A tendency toward lower NfL levels emerged from multivariable analysis in patients treated with remdesivir. |
| Senel et al. 2020 | Germany | CSF | CSF: ELISA; Serum: Simoa | Case report | Increased plasma NfL levels \( (58 \text{ pg/mL}) \). Increased plasma NfL levels at a second and third determination 7 and 23 after the lumbar puncture \( (61 \text{ and } 58 \text{ pg/mL respectively}) \). Increased pNfH levels \( (2131 \text{ pg/mL}) \) in the CSF analysis performed 27 days after COVID-19 symptoms onset \( (about \ two \ weeks \ after \ Miller-Fisher \ syndrome \ symptoms \ onset) \). Normal CSF t-tau and amyloid-beta-42 \( (265 \text{ and } 1109 \text{ pg/mL, respectively}) \). |
| Sun et al. 2021 | USA | Serum | R-plex Human NfL Kit (MSD) | Cross-sectional | Increased NfL concentration in serum neural enriched extracellular vesicles of all post-COVID-19 patients compared to historic controls. Increased amyloid beta, neurogranin, t-tau, p-tau181 in neural enriched extracellular vesicles of all post-COVID-19 patients compared to historic controls. |
| Sutter et al. 2021 | Switzerland | Serum | Simoa | Cross-sectional and prospective | 2.6 times higher serum NfL levels in the multivariate logistic analysis adjusted for age and pre-existing neurological comorbidities in patients with COVID-19 compared to non-COVID-19 patients \( (b=2.58; p=0.014) \). Serum NfL levels were 5.7 times higher in COVID-19 patients than in healthy controls \( (median \ 36.1, \text{ IQR} \ 20.0-55.9 \text{ pg/mL vs. } 6.3 \text{ pg/mL, } 4.7-8.5; p<0.001) \). |
Increased serum NfL levels were associated with a prolonged ICU and hospital stay and with prolonged mechanical ventilation.

Higher median serum NfL levels in patients with unfavourable vs. favourable outcome (55.9, IQR 38.7–95.7 pg/mL vs. 20.0, IQR 14.4–34.1 pg/mL; p<0.001)

Virhammar et al. 2020  
Sweden  CSF  CSF: in-house ELISA; plasma: Simoa  19 patients with COVID-19 related neurological complications (central and/or peripheral weakness n=8, altered mental status n=5, sensory symptoms n=1, confusion n=1, cranial nerve affection n=1, headache n=3) *†  Prospective  Increased CSF NfL levels above the age-adjusted reference range in 63% patients.

In 11 patients CSF NfL levels strongly correlated with plasma levels (r=0.98; p<0.001).

Increased CSF t-tau levels above the age-adjusted reference range in 37% patients.

Increased CSF GFAP levels above the age-adjusted reference range in 16% patients.

Strong correlation between CSF GFAP levels and plasma levels (r=0.97; p<0.001). For t-tau, no correlation could be demonstrated.

ALC: absolute lymphocyte count; β2M: β2-microglobulin; CI95: 95% confidence interval; CMAP: compound muscle activation potential; COVID-19: coronavirus disease 2019; CSA: muscle cross-sectional area; CSF: cerebrospinal fluid; CXCL13 = C-X-C motif chemokine ligand 13; ELISA: enzyme-linked immunoassorbent assay; GFAP: Glial Fibrillary Acidic protein; GCS: Glasgow coma scale; ICU: intensive care unit; IL: interleukin; IQR: interquartile range; LDH: lactate dehydrogenase; MSD: meso-scale discovery; NfL: Neurofilament light; PCR: polymerase chain reaction; pNfH: phosphorylated neurofilament high chain; pT18: phospho-tau 181; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SiMOA: single molecule array; TNFa: tumor necrosis factor alfa; TREM 2: riggering receptor expressed on myeloid cells 2; t-tau: total tau protein; UCH-L1: Ubiquitin C-terminal hydrolase L1; YKL-40: chitinase 3-like 1.

† age-matched reference dataset  
* age-matched control group  
** age-adjusted analysis, or age in control group did not differ statistically from patient group
## Supplementary table 3. NfL in psychiatric disorders

| Paper                        | Country of studied population | Examined biofluid | Examined biofluid | N                  | Study design                                      | NfL performance                                                                 |
|------------------------------|-------------------------------|-------------------|-------------------|--------------------|---------------------------------------------------|--------------------------------------------------------------------------------|
| Al Shweiki et al. 2019134    | Germany                       | Serum             | Simoa             | 11 bipolar patients, 11 schizophrenia patients, 28 patients with depression, 20 bvFTD 27 healthy controls. ** | Case control study                                                               | Schizophrenia, depression and bipolar patients did not show significant differences in the levels of serum NfL in comparison to controls. No difference between psychiatric subtypes. Serum NfL levels > 17.7 pg/ml differentiated bvFTD from schizophrenia with 100% sensitivity, 73% specificity and AUC 0.9 Serum NfL levels > 35.7 pg/ml distinguished bvFTD from depression with 70% sensitivity and 92.8%, specificity and AUC 0.89 Serum NfL levels > 26.55 pg/ml differentiated bvFTD from bipolar disorder with 80% sensitivity, 90.91% specificity and AUC 0.94 |
| Eratne et al. 2020135        | Australia                     | CSF               | ELISA (NF-light; UmanDiagnostics, Umea, Sweden) | 31 patients with primary psychiatric disorder: major depressive disorder (n=9), schizophrenia spectrum disorder (n=10), bipolar disorder (n=3), conversion disorder (n=3), somatic symptom disorder (n=3), adjustment disorder (n=2), anxiety disorder (n=1) and dissociative disorder (n=1); 77 patients with neurodegenerative or neurological disorder: Alzheimer’s disease (n=32), bvFTD (n=16), vascular dementia (n=3), dementia with Lewy bodies (n=2), Parkinson’s disease dementia (n=2), dementia not otherwise specified (n=11), corticobasal syndrome (n=4), multiple system atrophy (n=2), primary progressive aphasia (n=2), progressive supranuclear palsy (n=1), central nervous system vasculitis (n=1) and multiple sclerosis (n=1); 21 healthy controls ** | Cross-sectional retrospective study                                            | CSF NfL concentrations were significantly lower in patients with primary psychiatric disorder compared to patients with neurodegenerative or neurological disorder. |
| Fourier et al. 202079        | France                        | CSF               | ELISA (Uman Diagnostics, Umea, Sweden)          | 162 patients with neurodegenerative disorders: Alzheimer’s disease (n=49), FTD (n=50), definite Creutzfeldt–Jakob disease (n=20), Levy Body Dementia (n=26), Progressive Supranuclear Palsy (n=17); 64 patients with primary psychiatric disorder: depression (n=36), anxiety disorder (n=12), bipolar disorder (n=8), schizophrenia (n=3), post-traumatic disorders (n=2), other mental disorders (n=3). ** | Cross-sectional retrospective study                                            | CSF NfL concentrations were significantly lower in patients with primary psychiatric disorder compared to patients with neurodegenerative disorders. |
| Study                           | Country | Sample | Analysis Method | Participants                                                                 | Study Design | Findings                                                                 |
|--------------------------------|---------|--------|----------------|------------------------------------------------------------------------------|--------------|--------------------------------------------------------------------------|
| Gudmundsson et al. 2010         | Sweden  | CSF    | ELISA          | 13 patients with any depression: major depression disorder (n=11), dysthymia (n=2), 65 healthy controls ** | Cross-sectional study | CSF NfL concentrations were significantly higher in patients with major depression disorder compared to controls |
| Hellerhoff et al. 2021          | Germany | Serum  | Simoa          | 54 female patients with anorexia nervosa; 54 healthy controls *               | Case control study | Serum NfL concentrations were significantly higher in acutely underweight patients with anorexia nervosa compared to healthy control participants. Longitudinally, a decrease in NfL was observed in anorexia nervosa patients upon short-term partial weight restoration. |
| Jakobsson et al. 2014           | Sweden  | CSF    | ELISA          | 133 patients with bipolar disorder and 86 healthy control *                  | Case control study | CSF NfL concentrations significantly higher in patients with bipolar disorder compared to controls. |
| Katisko et al. 2020             | Finland | Serum  | Simoa          | 34 patients with primary psychiatric disorder: late onset schizophrenia (n=4), schizoaffective disorder (n=1), severe depression with psychotic symptoms (n=6), bipolar disorder with psychotic depression (n=1), persistent delusional disorder (n=4), unspecified non-organic psychosis (n=2), severe depression with psychotic symptoms (n=6), severe depression without psychotic symptoms (n=9), moderate depression without psychotic symptoms (n=4), and bipolar disorder (n=7); 91 patients with FTLD: bvFTD (n=66), nfvPPA (n=16), svPPA (n=4), FTLD- MND (n=5) ** | Case control study | Blood NfL levels were higher in the FTLD group compared to the group of patients with primary psychiatric disorders. No significant difference in NfL levels within the group of patients with primary psychiatric disorder. |
| Nilsson et al. 2020             | Sweden  | Plasma | Simoa          | 12 patients with anorexia nervosa; 11 patients with weight-recovered anorexia nervosa; 12 healthy controls * | Case control study | Plasma levels of NfL were significantly higher in patients with anorexia nervosa compared to both patients with weight-recovered anorexia nervosa and controls. |
| Rodrigues-Amorim et al. 2020   | Spain   | Plasma | ELISA          | 42 schizophrenia patients: 9 patients first-episode psychosis; 33 patients on treatment with aripiprazole (n=8), risperidone (n=8), olanzapine (n=8); 40 healthy controls * | Case control study | Blood NfL levels were elevated in patients with schizophrenia compared to healthy controls. |
| Rolstad et al. 2015<sup>169</sup> | Sweden | CSF | ELISA (NF-light, UmanDiagnostics AB, Umeå, Sweden). | 82 patients with bipolar disorder and 71 healthy controls * | Case control study | CSF NfL concentrations were significantly higher in patients with bipolar disorder compared to controls. |
|----------------------------------|--------|-----|-------------------------------------------------|-------------------------------------------------|---------------------|-------------------------------------------------|
| Vijverberg et al. 2017<sup>184</sup> | Netherland | CSF | ELISA (Uman) | 25 patients with primary psychiatric disorder: major/minor depression (n=11), bipolar disorder (n=4), autism spectrum (n=1), anxiety disorder (n=1), obsessive-compulsive disorder (n=1), and personality disorder (n=7), 22 with bvFTD n=22 ** | Case control study | CSF NfL concentrations were significantly lower in patients with primary psychiatric disorder compared to patients with bvFTD. |
| Wentz et al. 2020<sup>76</sup> | Sweden | Serum | Simoa | 51 patients with teenage-onset of anorexia nervosa; 51 healthy controls * | Case control study, prospective follow up for 30 years. | Serum NfL concentrations were significantly higher in the group of patients with anorexia nervosa compared to the control group. |

FTLD: frontotemporal lobar degeneration; bvFTD: behavioral variant of frontotemporal dementia; nfvPPA: non fluent variant of frontotemporal dementia; svPPA: semantic variant of frontotemporal dementia; FTLD- MND: frontotemporal lobar degeneration with motoneuron disease

<sup>†</sup> age-matched reference dataset
<sup>‡</sup> age-matched control group
<sup>**</sup> age-adjusted analysis, or age in control group did not differ statistically from patients group
**Neurofilament light chain protein in other non-primary neurological conditions**

**Gynecological diseases**

Preeclampsia represents the most important hypertensive disorder in pregnancy affecting between 2% and 8% of all pregnant women.\(^1\) It is defined by the new onset of hypertension and proteinuria after 20 weeks of gestation with or without multisystem symptoms, such as neurological manifestations (headache, altered consciousness, visual disturbances), epigastric pain with nausea and vomit, thrombocytopenia and abnormal liver enzyme (HELLP syndrome).\(^2,3\) Additionally, women with preeclampsia showed a higher risk to develop cerebrovascular diseases, dementia and seizures in later life.\(^2,4-6\)

Thus, the identification of a reliable biomarker reflecting the degree of central nervous system insult might help to directly estimate the cerebral effects of preeclampsia, preferably before neurological complications occur. In a prospective longitudinal study Evers et al. firstly disclosed elevated levels of serum NfL in women with preeclampsia compared to women with normal pregnancy.\(^7\) Interestingly, serum NfL concentration positively correlated with maternal age, number of pregnancies and proteinuria and independently predicted the development of preeclampsia in women older than 36 with a similar or even superior accuracy to that of well-established preeclampsia biomarkers, such as soluble fms-like tyrosine kinase (sFlt-1) and placental growth factor (PIGF).\(^7,8\)

The observation that NfL was the only biomarker to remain significantly higher at delivery either in CSF or serum after adjusting for possible confounders (parity and BMI), may strongly support the reliability of the marker.\(^9\)

**Disorders due to physical agents**

Decompression sickness (DCS) is a condition resulting from the oo rapid diver ascending towards the surface, determining the formation of venous gas emboli (VGE) made of previously solubilized nitrogen.\(^10\) Circulating VGE may occlude arteries by passing through either cardiac or lung veno-arterial shunts and provoking a wide range of clinical symptoms, such as itchy skin, fatigue, pain and neurological manifestations (stroke, seizures, coma).\(^11\) Apart from VGE, vascular effects, exposure to high environment pressure may be responsible of the so called high-pressure neurological syndrome, a neuromuscular dysfunction characterized by with headache, nausea, dizziness, tremor and myoclonus.\(^10\) Notwithstanding these neurological implications, different studies failed to report significant changes in serum and/or CSF NfL concentrations after diving \(^12,13\) or prolonged exposure to dry hyperbaric chamber.\(^14\) As
concern high-pressure neurological syndrome, biomarkers of neuro-axonal injury have never been evaluated so far in this condition.

Headache accompanied by anorexia, nausea, dizziness, fatigue, may also be part of the so-called acute mountain sickness (AMS) syndrome as a result from the exposure to hypobaric hypoxia.\textsuperscript{15,16} AMS-related symptoms are usually reversible but sometimes can worsen and lead to the development of high-altitude cerebral edema.\textsuperscript{15} In this regard, Sareban et al. disclosed significantly increased serum NfL levels in healthy subjects after rapid ascending from baseline to high altitude (4559 m).\textsuperscript{17} This increase was not related to the magnitude of hypoxemia nor to the occurrence of AMS, suggesting that AMS pathophysiology is largely independent from neuroaxonal integrity.

### Dermatological conditions

Serum NfL and tau concentrations were found to be significantly higher in patients with plaque-type psoriasis when compared to healthy controls, especially in patients with disease onset less than 40 years.\textsuperscript{18} Furthermore, their concentrations correlated with the disease severity of the cutaneous manifestations as assessed by the Psoriasis Area Severity Index score.\textsuperscript{18} These findings may support the hypothesis of an increased risk of cognitive decline associated with this dermatologic condition, as suggested by some recent studies.\textsuperscript{19,20}
Supplementary References

1. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. The Lancet. 2010;376(9741):631-644. doi:10.1016/S0140-6736(10)60279-6
2. Roberts JM, Redman CWG. Pre-eclampsia: more than pregnancy-induced hypertension. The Lancet. 1993;341(8858):1447-1451. doi:10.1016/0140-6736(93)90889-O
3. Tranquilli AL. Early and late-onset pre-eclampsia. Pregnancy Hypertension: An International Journal of Women’s Cardiovascular Health. 2014;4(3):241. doi:10.1016/j.preghy.2014.04.007
4. Siepmann T, Boardman H, Bilderbeck A, et al. Long-term cerebral white and gray matter changes after preeclampsia. Neurology. 2017;88(13):1256-1264. doi:10.1212/WNL.0000000000003765
5. Fields JA, Garovic VD, Mielke MM, et al. Preeclampsia and cognitive impairment later in life. American Journal of Obstetrics and Gynecology. 2017;217(1):74.e1-74.e11. doi:10.1016/j.ajog.2017.03.008
6. Bushnell C, Chireau M. Preeclampsia and Stroke: Risks during and after Pregnancy. Stroke Research and Treatment. 2011;2011:1-9. doi:10.4061/2011/858134
7. Evers KS, Atkinson A, Barro C, et al. Neurofilament as Neuronal Injury Blood Marker in Preeclampsia. Hypertension. 2018;71(6):1178-1184. doi:10.1161/HYPERTENSIONAHA.117.10314
8. Bergman L, Zetterberg H, Kaibola H, Hagberg H, Blennow K, Åkerud H. Blood-based cerebral biomarkers in preeclampsia: Plasma concentrations of NfL, tau, S100B andNSE during pregnancy in women who later develop preeclampsia - A nested case control study. PLOS ONE. 2018;13(5):e0196025. doi:10.1371/journal.pone.0196025
9. Andersson M, Oras J, Thörn SE, et al. Signs of neuroaxonal injury in preeclampsia—A case control study. PLOS ONE. 2021;16(2):e0246786. doi:10.1371/journal.pone.0246786
10. Edmonds C, Bennett M, Lippmann J, Mitchell S. Diving and Subaquatic Medicine. CRC Press; 2015. doi:10.1201/b18700
11. Mitchell SJ, Bennett MH, Moon RE. Decompression Sickness and Arterial Gas Embolism. New England Journal of Medicine. 2022;386(13):1254-1264. doi:10.1056/NEJMra2116554
12. Shahim P, Arnell P, Kvarnström A, et al. Cerebrospinal fluid markers of central nervous system injury in decompression illness - a case-controlled pilot study. Diving Hyperb Med. 2015;45(4):240-243.
13. Rosén A, Oscarsson N, Kvarnström A, et al. Serum tau concentration after diving – an observational pilot study. Diving and Hyperbaric Medicine Journal. 2019;49(2):88-95. doi:10.28920/dhm49.2.88-95
14. Rosén A, Gennser M, Oscarsson N, et al. Biomarkers of neuronal damage in saturation diving—a controlled observational study. European Journal of Applied Physiology. 2020;120(12):2773-2784. doi:10.1007/s00421-020-04499-y
15. Bärttsch P. Acute High-Altitude Illnesses. New England Journal of Medicine. 2013;369(17):1664-1667. doi:10.1056/NEJMc1309747
16. Bartsch P, Bailey DM, Berger MM, Knauth M, Baumgartner RW. Acute Mountain Sickness: Controversies and Advances. High Altitude Medicine & Biology. 2004;5(2):110-124. doi:10.1089/1527029041352108
17. Sareban M, Berger MM, Pinter D, et al. Serum neurofilament level increases after ascent to 4559 m but is not related to acute mountain sickness. *European Journal of Neurology*. 2021;28(3):1004-1008. doi:10.1111/ene.14606

18. Ökan G, Baki AM, Yorulmaz E, Doğru-Abbasoğlu S, Vural P. A preliminary study about neurofilament light chain and tau protein levels in psoriasis: Correlation with disease severity. *Journal of Clinical Laboratory Analysis*. 2021;35(1). doi:10.1002/jcla.23564

19. Gisondi P, Sala F, Alessandrini F, et al. Mild Cognitive Impairment in Patients with Moderate to Severe Chronic Plaque Psoriasis. *Dermatology*. 2014;228(1):78-85. doi:10.1159/000357220

20. Lin CC, Lin HC, Chiu HW. Association Between Psoriasis and Dementia: A Population-Based Case–Control Study. *American Journal of Clinical Dermatology*. 2019;20(3):457-463. doi:10.1007/s40257-018-00420-8