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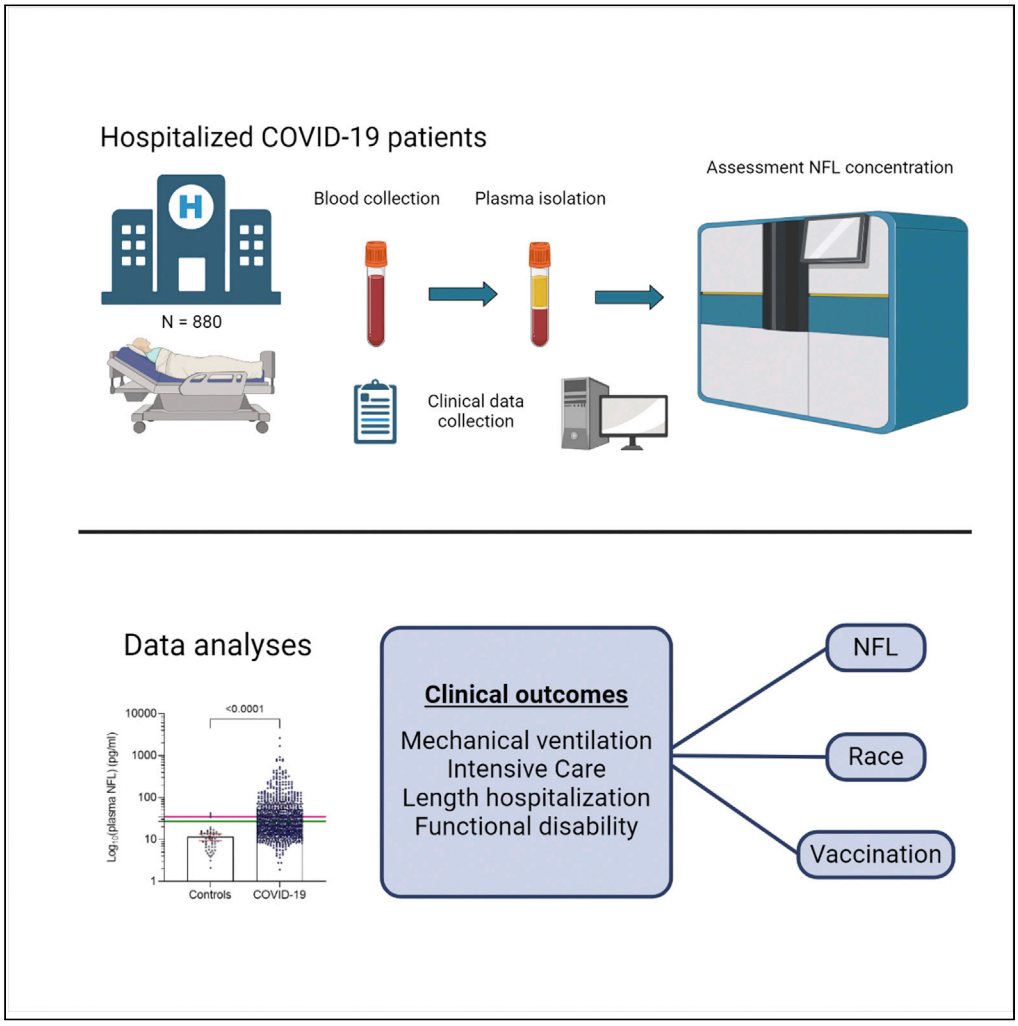

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Highlights

Plasma NFL is elevated in patients with COVID-19 early during their hospitalization

Higher NFL levels associate with greater functional disability at discharge

Clinical outcomes evaluated in patients with COVID-19 do not differ by race

Vaccination associates with less disability at the time of hospital discharge

Erben et al., iScience 25, 105272
November 18, 2022
https://doi.org/10.1016/j.isci.2022.105272

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Neurofilament light chain and vaccination status associate with clinical outcomes in severe COVID-19

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SUMMARY

Blood neurofilament light chain (NFL) is proposed to serve as an estimate of disease severity in hospitalized patients with coronavirus disease 2019 (COVID-19). We show that NFL concentrations in plasma collected from 880 patients with COVID-19 within 5 days of hospital admission were elevated compared to controls. Higher plasma NFL associated with worse clinical outcomes including the need for mechanical ventilation, intensive care, prolonged hospitalization, and greater functional disability at discharge. No difference in the studied clinical outcomes between black/African American and white patients was found. Finally, vaccination associated with less disability at time of hospital discharge. In aggregate, our findings support the utility of measuring NFL shortly after hospital admission to estimate disease severity and show that race does not influence clinical outcomes caused by COVID-19 assuming equivalent access to care, and that vaccination may lessen the degree of COVID-19-caused disability.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused over 1 million coronavirus disease 2019 (COVID-19) deaths in the United States (Coronavirus Resource Center. John Hopkins University). SARS-CoV-2 infections can cause adverse events affecting multiple organs (Lopes-Pacheco et al., 2021) with some patients developing short- and long-term neurological symptoms, such as headaches, dizziness, ageusia, anosmia, seizures, strokes, and cognitive dysfunction (Fernandez-de-Las-Penas et al., 2022). Given these neurological manifestations, we recently measured neurofilament light chain (NFL), a marker of neuroaxonal injury with prognostic utility for various neurodegenerative diseases (Khalil et al., 2018), in patients with severe COVID-19 (Prudencio et al., 2021). Our prior study, in which NFL was measured in serum collected longitudinally from 142 hospitalized patients with COVID-19 (Prudencio et al., 2021), and similar investigations conducted by others (Aamodt et al., 2021; Espindola et al., 2021; Garcia et al., 2021; Guasp et al., 2022; Kanberg et al., 2020; Masvekar et al., 2022; Pilotto et al., 2021; Virhammar et al., 2021), established that elevated blood NFL associates with worse clinical outcomes including the need for mechanical ventilation, intensive care, prolonged hospitalization, and greater functional disability at discharge. In the present study, we sought to validate our prior observations supporting the use of blood NFL as a disease severity marker, and to evaluate whether NFL concentrations in blood collected from patients with COVID-19 early upon their admission to hospital may also inform clinical outcome severity. We additionally availed data from our large cohort of 880 patients hospitalized with COVID-19 to probe important unresolved questions, including whether race and vaccination status influence clinical outcomes in patients hospitalized for COVID-19. Recent publications have postulated racial disparities in outcomes after SARS-CoV-2 infection (Grosicki et al., 2022; Pathak et al., 2022; Qeadan et al., 2021; Walls et al., 2022), but there remains a need for additional rigorous studies to parse out inconsistency in findings. Furthermore, vaccination has been postulated to prevent severe disease and need for hospitalization (Moline et al., 2021; Thompson et al., 2021). However, it is unclear whether patients who are vaccinated, but who must nonetheless be hospitalized for COVID-19, fare better than unvaccinated individuals hospitalized for COVID-19.

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https://doi.org/10.1016/j.isci.2022.105272

Table 1. Characteristics and outcomes of healthy controls and individuals hospitalized for COVID-19

Variable	N	Median (minimum, maximum) or No. (%)
Controls		
Age at collection (years)	55	63 (31, 87)
Sex (Male)	55	28 (50.9%)
Race	52	
White		46 (88.5%)
Black/African American		4 (7.7%)
Asian		1 (1.9%)
Other		1 (1.9%)
COVID-19 patients		
Age at admission/collection (years)	880	60 (21, 102)
Sex (Male)	880	515 (58.5%)
Race	862	
White		692 (80.3%)
Black/African American		118 (13.7%)
Asian		33 (3.8%)
Other		19 (2.2%)
Body mass index (BMI)	874	29.5 (15.3, 84.7)
Obesity	874	406 (46.5%)
Chronic kidney disease (CKD)	880	229 (26.0%)
Vaccination status	880	
Fully vaccinated		159 (18.1%)
Partially vaccinated		48 (5.5%)
Unvaccinated		673 (76.5%)
Length of time from admission to blood draw (days)	880	
0		30 (3.4%)
1		110 (12.5%)
2		497 (56.5%)
3		216 (24.5%)
4		18 (2.0%)
5		9 (1.0%)
Charlson comorbidity index score	880	
0		542 (61.6%)
1		64 (7.3%)
2		63 (7.2%)
3		42 (4.8%)
4		28 (3.2%)
≥5		141 (16.0%)

RESULTS

Patient characteristics

From June 2020 to October 2021, we collected plasma from patients hospitalized at the Mayo Clinic in Florida due to COVID-19. In the present cross-sectional study, NFL concentrations were measured in plasma samples collected from 880 patients within 5 days of hospital admission. For comparison, NFL concentrations were measured in plasma obtained from 55 non-hospitalized healthy individuals prior to the COVID-19 pandemic (from February 2011 to July 2019). A summary of individual characteristics and clinical outcomes is provided in Table 1. The median age of patients with COVID-19 was 60 years (range: 21–102), and 58.5% of them were male (N = 515). Control individuals had a median age of 63 years (range: 31–87),

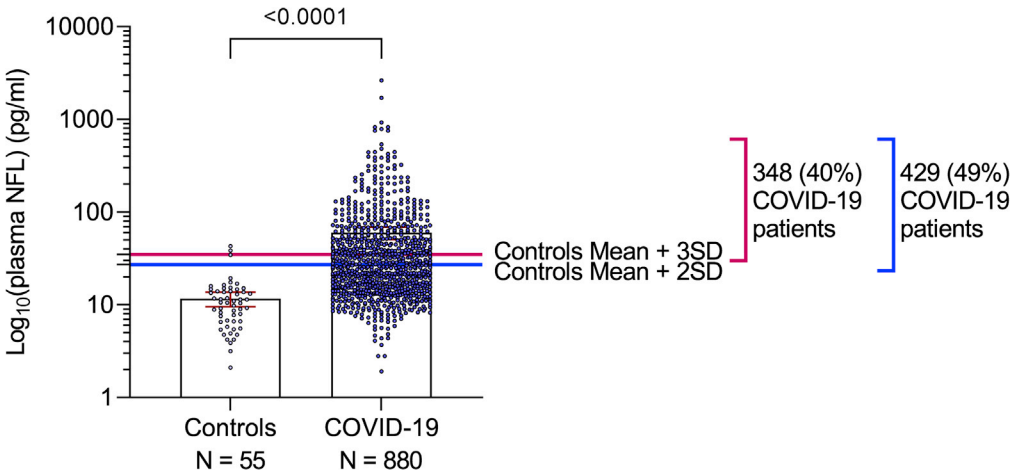

Figure 1. Plasma NFL is elevated in patients with COVID-19 within 5 days of hospitalization

Comparison of plasma NFL concentrations between healthy controls (Controls, N = 55) and hospitalized patients with COVID-19 (N = 880). Bars represent mean NFL concentrations with 95% confident intervals (CIs). Statistical differences were assessed using a stratified van Elteren Wilcoxon rank-sum test, where the test was stratified by both age as a four-level categorical variable (based on sample quartiles) and sex. ****$p < 0.0001$. Mean NFL concentration in the control group +2 or +3 standard deviations (SD) are respectively shown by a solid green or pink horizontal line, respectively. NFL concentrations are shown on the base 10 logarithm scale.

and 50.9% were male (N = 28). Our cohort included 80.3% (N = 692) white, 13.7% black/African American (N = 118), 3.8% Asian (N = 33), and 2.2% other (N = 19). Most patients with COVID-19 (76.5%, N = 673) were unvaccinated, with the remainder being either partially vaccinated (5.5%, N = 48 with one dose of Moderna or Pfizer-BioNTech) or fully vaccinated (18.1%, N = 159 with two doses of Moderna or Pfizer-BioNTech, or 1 dose Janssen/Johnson & Johnson). Several patients with COVID-19 (61.6%, N = 542) had no other comorbidities; however, a significant proportion of patients (38.4%, N = 338) had a Charlson comorbidity index (Charlson et al., 1987, 1994) score of 1 or higher, with 141 patients (16%) having a score ≥ 5 reflecting the high rates of comorbidities in this population (Table 1).

Plasma NFL is elevated in patients with COVID-19 within 5 days of their hospitalization

We previously found that serum NFL was elevated during the hospitalization of 142 patients with COVID-19 (Prudencio et al., 2021). Consistent with these prior data, plasma NFL was significantly higher in our cohort of patients with COVID-19 (median 25.8 pg/mL, range: 1.9–2,641.2 pg/mL, N = 880), for which plasma was collected within 5 days of hospital admission (and before discharge), compared to controls (median 10.5 pg/mL, range: 2.1–43.0 pg/mL, N = 55; Figure 1) in unadjusted analyses ($p < 0.0001$) and analyses adjusted for age and sex ($p < 0.0001$) (Table S1). Among all patients with COVID-19, 48.8% (N = 429) and 39.6% (N = 348), respectively, had plasma NFL concentrations greater than 2 or 3 standard deviations above the mean NFL concentration of control individuals (Figure 1). This finding was in line with that of our prior study in which 53% and 34% of patients with COVID-19, respectively, had a maximum serum NFL concentration 2 or 3 standard deviations above the mean NFL concentration of controls (Prudencio et al., 2021).

Higher plasma NFL associates with worse clinical outcomes in patients hospitalized for COVID-19

We next examined whether we could replicate our prior findings showing strong relationships between higher serum NFL concentrations and worse clinical outcomes (Prudencio et al., 2021). In our original study, NFL concentrations were measured in serum collected longitudinally from each patient throughout their hospitalization (Prudencio et al., 2021). To better model the intended use of NFL as a marker of COVID-19 severity, in the present cross-sectional study, we measured NFL concentrations in plasma collected within 5 days of hospital admission. In the cohort of 880 hospitalized individuals with COVID-19, we found that plasma NFL concentrations were significantly higher in patients who were intubated, admitted to the intensive care unit (ICU), had a longer lengths of stay (LOS), or had a higher modified Rankin scale (mRS) score (an indicator of functional impairment) (Nunn et al., 2016) at discharge both in unadjusted analysis

Table 2. Associations between plasma NFL concentrations and clinical outcomes

Association between NFL and	Median (minimum, maximum)	NFL level	Unadjusted analysis		Adjusting for age, sex, BMI, CKD, race, vaccination status, and Charlson comorbidity index score	
			Estimate (95% CI)	p value	Estimate (95% CI)	p value
Intubation	No intubation (N = 796) 25.4 (1.9, 2641.2)	Intubation (N = 84) 35.2 (3.7, 1707.9)	0.37 (0.05, 0.69)	0.023	0.39 (0.13, 0.66)	0.004
ICU admission	No ICU admission (N = 737) 24.7 (1.9, 2641.2)	ICU admission (N = 143) 36.1 (3.7, 1707.9)	0.49 (0.23, 0.74)	<0.001	0.38 (0.17, 0.60)	<0.001
Length of hospital stay (days)	LOS ≤5 days (N = 449) 22.2 (1.9, 2641.2)	LOS >5 days (N = 431) 32.1 (2.8, 1707.9)	0.13 (0.05, 0.20)	0.002	0.09 (0.03, 0.16)	0.006
mRS at discharge (>3)	mRS at discharge ≤3 (N = 763) 24.0 (1.9, 923.4)	mRS at discharge >3 (N = 117) 49.4 (4.8, 2641.2)	0.14 (0.09, 0.19)	<0.001	0.05 (0.01, 0.10)	0.022

CI, confidence interval; LOS, length of hospital stay; ICU, intensive care unit; mRS, modified Rankin scale, CKD, chronic kidney disease; BMI, body mass index. For descriptive summaries of NFL levels, for ease of presentation LOS was categorized using the sample median, while mRS at discharge was categorized using a pre-defined cutoff of interest. Associations of intubation, ICU admission, length of hospital stay, and mRS at discharge (all as independent variables) with NFL level (as the dependent variable) were evaluated using linear regression models. Regression coefficients are interpreted as the change in the mean NFL outcome measure (on the base 2 logarithm scale) corresponding to presence of the given characteristic (intubation and ICU admission), to each doubling of length of hospital stay, and to each 1-unit increase in mRS at discharge. Given that intubation, ICU admission, LOS, and mRS at discharge occurred either prior to measurement of some NFL values (intubation or ICU admission) or at a very similar time point as measurement of some NFL values (intubation, ICU admission, LOS, and mRS at discharge), performing an analysis that examined the ability of NFL to predict these outcome measures was not possible given the data. For this reason, we assessed associations between these four variables and NFL level when considering NFL level as the dependent variable to avoid any implication regarding the predictive utility of NFL for these four outcomes. p-values < 0.05 (given that the lowest three p values were lower than the 0.0125, 0.0167, and 0.025 significance thresholds of the step-down correction) were considered as statistically significant after applying a Holm step-down correction for multiple testing in multivariable analysis.

and analysis adjusted for age, sex, body mass index (BMI), presence of chronic kidney disease (CKD), race, vaccination status, and Charlson comorbidity index score (Table 2). Similar findings were noted when additionally adjusting for time from admission to blood draw (Table S2), a potential confounder based on our prior observation that serum NFL positively associates with time from hospital admission to blood draw (Prudencio et al., 2021).

Clinical outcomes in patients hospitalized for COVID-19 do not differ by race

Within the COVID-19 cohort, 118 patients were black/African American and 692 where white, thus allowing comparisons of clinical characteristics and outcomes between these two races. Compared to the group of white patients, the group of black/African American patients was significantly younger and had a significantly lower percentage of males. In addition, the BMI of black/African American patients was significantly higher, and their CKD status was significantly worse, than those of white patients (Table S3). The proportion of fully vaccinated black/African American patients was higher than that of white patients, but they presented with more severe comorbidity, as assessed using the Charlson comorbidity index (Table S3). Nevertheless, clinical outcomes, including the need for intubation, ICU admission, LOS, or mRS score, at discharge did not differ between white and black/African American patients either in unadjusted analysis or analysis adjusted for age, sex, BMI, vaccination status, and Charlson comorbidity index score (Table 3). However, a trend of higher plasma NFL in black/African American patients compared to white patients was observed in analyses adjusted for the above-mentioned confounders ($p = 0.013$; $p < 0.01$ was considered significant after correction for multiple testing, Table 3). These results remained consistent when also adjusting for time from admission to blood draw (Table S4).

Functional impairment at discharge is milder in vaccinated patients with COVID-19

Most hospitalized patients with COVID-19 were unvaccinated (76.5%, N = 673) with only 18.1% of patients being fully vaccinated (N = 159). No difference in plasma NFL levels, the need for intubation, ICU admission, or LOS was observed between unvaccinated and vaccinated individuals either in unadjusted analyses or in analysis adjusting for age, sex, BMI, CKD, race, and Charlson comorbidity index score (Table 4). However, fully vaccinated individuals who, incidentally, were older than unvaccinated patients and more

Table 3. Comparison of outcomes and NFL concentrations between white and black/African American patients with COVID-19

	Median (minimum, maximum) or No. (%) of patients		Comparison of the given variable between black/African American patients and white patients (the reference group)				
				Unadjusted analysis		Adjusting for age, sex, BMI, CKD, vaccination status, and Charlson comorbidity index score	
Outcome	White patients (N = 692)	Black/African American patients (N = 118)	Association measure	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Intubation	68 (9.8%)	8 (6.8%)	Odds ratio	0.67 (0.31, 1.43)	0.30	0.60 (0.27, 1.34)	0.21
ICU admission	103 (14.9%)	23 (19.5%)	Odds ratio	1.39 (0.84, 2.29)	0.20	1.31 (0.76, 2.26)	0.33
Length of hospital stay (days)	5.0 (1.0, 193.0)	5.0 (1.0, 61.0)	Multiplicative effect on mean	0.92 (0.77, 1.09)	0.31	0.93 (0.77, 1.12)	0.42
mRS at discharge (>3)	94 (13.6%)	14 (11.9%)	Odds ratio	0.81 (0.57, 1.15)	0.24	1.12 (0.76, 1.64)	0.57
Plasma NFL level (pg/mL)[a]	25.6 (1.9, 2641.2)	28.6 (2.8, 1707.9)	Regression coefficient	0.19 (−0.09, 0.47)	0.18	0.32 (0.07, 0.57)	0.013

CI, confidence interval; LOS, length of hospital stay; ICU, intensive care unit; mRS, modified Rankin scale, CKD, chronic kidney disease; BMI, body mass index. mRS = modified Rankin scale at discharge was categorized using a pre-defined cutoff of interest for descriptive summaries only and was analyzed as an ordinal variable in all association analysis. Comparisons of outcomes between black/African American and white patients were made using binary logistic regression models (intubation and ICU admission), negative binomial regression models (length of hospital stay), proportional odds logistic regression models (mRS at discharge), and linear regression models (plasma NFL). For intubation and ICU admission, odds ratios are interpreted as the multiplicative increase in the odds of the given outcome for black/African American patients compared to white patients. For length of hospital stay, the multiplicative effect on the mean is interpreted as the multiplicative increase in mean length of hospital stay for black/African American patients compared to white patients. For mRS at discharge, the odds ratio is interpreted as the multiplicative increase in the odds of a higher mRS at discharge for black/African American patients compared to white patients. For plasma NFL, the regression coefficient is interpreted as the difference in mean plasma NFL level (on the base 2 logarithm scale) for black/African American patients compared to white patients. p-values < 0.01 were considered as statistically significant after applying a Holm step-down correction for multiple testing in multivariable analysis.

[a]Analysis for plasma NFL level was also adjusted for time from admission to blood draw.

frequently had CKD and higher Charlson comorbidity index scores (Table S5), presented with significantly less functional impairment based on their mRS score at discharge in both unadjusted analysis and analysis adjusted for potential confounders (Tables 4 and S6). Of interest, following discharge from hospital, 53 of the patients with COVID-19 died, the majority of whom (85%, N = 45) were unvaccinated.

DISCUSSION

We show that plasma NFL was significantly higher in patients with COVID-19 compared to controls, with approximately 50% of patients having an NFL concentration greater than 2 standard deviations above the mean NFL concentration of the control group. We additionally observed that higher plasma NFL correlated with worse clinical outcomes. Not only do these findings validate those from our prior study (Prudencio et al., 2021) but we also established that elevated NFL levels can be detected soon after hospitalization. Therefore, measuring NFL shortly after admission to hospital to estimate disease severity may inform recommendations for more aggressive interventions. It is also intriguing to speculate that high plasma NFL may foretell the development of long-term neurological symptoms. As such, monitoring patients who had elevated plasma NFL while in hospital for neurologic signs emerging after their discharge may be warranted. Such longitudinal studies may also inform whether COVID-19-associated neuroaxonal injury involves the central and/or the peripheral nervous systems.

In our COVID-19 cohort, we noted no difference in clinical outcomes between black/African American and white patients despite the former having worse Charlson comorbidity index scores, and a trend of higher plasma NFL. In contrast to our findings, Qeadan and colleagues reported that black/African American patients with COVID-19 had worse clinical outcomes, such as longer LOS and need for intubation, compared

Table 4. Comparison of outcomes and NFL concentrations between fully vaccinated patients and unvaccinated patients with COVID-19

	Median (minimum, maximum) or No. (%) of patients		Comparison of the given variable between unvaccinated patients and fully vaccinated patients (the reference group)				
				Unadjusted analysis		Adjusting for age, sex, BMI, CKD, race, and Charlson comorbidity index score	
Outcome	Fully vaccinated patients (N = 159)	Unvaccinated patients (N = 673)	Association measure	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Intubation	12 (7.5%)	68 (10.1%)	Odds ratio	1.38 (0.73, 2.61)	0.33	1.40 (0.71, 2.73)	0.33
ICU admission	21 (13.2%)	116 (17.2%)	Odds ratio	1.37 (0.83, 2.26)	0.22	1.53 (0.90, 2.59)	0.11
Length of hospital stay (days)	5.0 (1.0, 193.0)	5.0 (1.0, 178.0)	Multiplicative effect on mean	1.11 (0.95, 1.30)	0.18	1.01 (0.86, 1.19)	0.88
mRS at discharge (>3)	12 (7.5%)	99 (14.7%)	Odds ratio	1.57 (1.15, 2.15)	0.005	2.06 (1.47, 2.89)	<0.001
Plasma NFL level (pg/mL)[a]	40.1 (2.8, 785.0)	23.7 (1.9, 2641.2)	Regression coefficient	−0.40 (−0.64, −0.15)	0.002	0.15 (−0.07, 0.36)	0.18

CI, confidence interval; LOS, length of hospital stay; ICU, intensive care unit; mRS, modified Rankin scale, CKD, chronic kidney disease; BMI, body mass index. mRS = modified Rankin scale at discharge was categorized using a pre-defined cutoff of interest for descriptive summaries only and was analyzed as an ordinal variable in all association analysis. Comparisons of outcomes between fully vaccinated and non-vaccinated patients were made using binary logistic regression models (intubation and ICU admission), negative binomial regression models (length of hospital stay), proportional odds logistic regression models (mRS at discharge), and linear regression models (plasma NFL). For intubation and ICU admission, odds ratios are interpreted as the multiplicative increase in the odds of the given outcome for non-vaccinated patients compared to fully vaccinated patients. For length of hospital stay, the multiplicative effect on the mean is interpreted as the multiplicative increase in mean length of hospital stay for non-vaccinated patients compared to fully vaccinated patients. For mRS at discharge, the odds ratio is interpreted as the multiplicative increase in the odds of a higher mRS at discharge for non-vaccinated patients compared to fully vaccinated patients. For plasma NFL, the regression coefficient is interpreted as the difference in mean plasma NFL level (on the base 2 logarithm scale) for non-vaccinated patients compared to fully vaccinated patients. p-values < 0.0125 were considered as statistically significant after applying a Holm step-down correction for multiple testing in multivariable analysis.

[a]Analysis for plasma NFL level was also adjusted for time from admission to blood draw.

to white patients with comparable scores on the Elixhauser comorbidity index (Qeadan et al., 2021); like the Charlson comorbidity index, the Elixhauser index assesses the burden of comorbidities based on hospital data (Baron et al., 2020; Chang et al., 2016). Discrepancies among findings in the Qeadan study and ours could be due, in part, to the possibility that neither of these comorbidity indices is well suited to patients with COVID-19; indeed, Monterde et al. suggested that a different method to assess comorbidities would be necessary in this patient population (Monterde et al., 2021). It also bears mentioning that, in the Qeadan study, not all individuals were hospitalized and information on vaccination status, which is known to influence disease severity, was lacking. Finally, social and economic factors that influence the timely access to proper medical care needed to manage severe COVID-19 (Espindola et al., 2021; Garcia et al., 2021; Kanberg et al., 2020) also likely explain differences in findings between the two studies. Whereas patients with COVID-19 in the Qeadan study were from various geographic regions, assessed at different hospitals, and likely had a wide range of social and economic conditions, our cohort was likely more homogeneous as it was limited to hospitalized patients with COVID-19 receiving care at the Mayo Clinic in Jacksonville Florida.

Most hospitalized patients with COVID-19 in our study were unvaccinated; however, those who were fully vaccinated were not less likely to require intubation, be admitted to the ICU, or experience a shorter LOS. We did nonetheless observe that, at discharge, fully vaccinated patients had a lower mRS score, indicative of a better functional status, than unvaccinated patients. Thus, vaccination not only decreases the risk of being hospitalized for COVID-19 (Moline et al., 2021; Thompson et al., 2021) but also lessens the degree of COVID-19-caused disability.

Our study has several strengths, including evaluating plasma NFL collected early after hospital admission in the largest number of patients with COVID-19. We also undertook a rigorous statistical approach when examining plasma NFL as a marker of COVID-19 severity, and when investigating associations of plasma

NFL, vaccination status, and racial differences with clinical outcomes. By evaluating only patients with COVID-19 hospitalized at the Mayo Clinic in Florida, we focused on a group of individuals likely to have similar social, economic, and environmental determinants of health. By doing so, our findings, in aggregate with those of others (Brakefield et al., 2022; Erben et al., 2022; Grosicki et al., 2022; Qeadan et al., 2021; Walls et al., 2022), suggest that racial disparities likely influence clinical outcomes caused by COVID-19 more so than race itself.

In summary, our studies support the utility of measuring plasma NFL soon after hospitalization for SARS-CoV-2 infection to estimate disease severity and consequently inform patient care. We also found no difference in the studied clinical outcomes between black/African American and white patients, suggesting that current treatment measures for managing SARS-CoV-2 infection are similarly effective for both races assuming equivalent access to care. Finally, in addition to decreasing the risk of being hospitalized for COVID-19, we show that vaccination associates with a lower degree of disability experienced by patients with COVID-19 at time of their discharge from hospital.

Limitations of the study

When examining clinical outcomes between white and black/African American patients, we did not adjust for social, economic, or environmental determinants of health. Our study also has other limitations. Although the number of black/African American patients (N = 118) was relatively high, it was nonetheless lower than the number of white patients (N = 692), and thus there is a possibility that some analyses involving race were underpowered. In addition, we could not consider other races and ethnic groups due to the small size of these cohorts, nor did we consider the strain of SARS-CoV-2 virus infecting each patient since only a small percentage of hospitalized patients underwent viral genomic studies. Furthermore, imaging data that could confirm the degree of neuronal damage predicted from NFL measurements was lacking. Consequently, follow-up studies would benefit from imaging measures as well as other markers of neuronal injury and/or inflammation as they could provide mechanistic insight on the relationship between NFL and COVID-19.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105272.

ACKNOWLEDGMENTS

We thank the Mayo Clinic COVID-19 Task Force for their support in conducting this research study. We are grateful to all individuals who donated samples. We thank Dr. Sarah Pickles who aided with the generation of the graphical abstract, created with BioRender.com. We thank Ms. Piper Burrow for her assistance in

sample processing. This work was supported by the National Institutes of Health [R35NS097273 (L.P.); P01NS084974 (T.F.G., B.O., L.P.); P01NS099114 (T.F.G., L.P.); U54NS123743 (M.P., L.P.); RF1AG062077 (L.P.); RF1AG062171 (L.P.); RF1NS120992 (M.P.), Mayo Clinic Foundation (L.P.), Robert Packard Center for ALS Research at Johns Hopkins (L.P.). Z.K.W. is partially supported by the NIH/NIA and NIH/NINDS (1U19AG063911, FAIN: U19AG063911), Mayo Clinic Center for Regenerative Medicine, the gifts from the Donald G. and Jodi P. Heeringa Family, the Haworth Family Professorship in Neurodegenerative Diseases fund, and The Albertson Parkinson's Research Foundation.

AUTHOR CONTRIBUTIONS

Conceptualization, Y.M.E., M.P., J.F.M., and L.P. Resources, Y.M.E., M.P., C.P.M., K.R.J.-W., J.A.D., C.N.C., M.T.L., N.Q., Y.S., R.H.A., L.M.D., J.L.B., G.S.D., B.O., K.A.N., Z.K.W., and J.B.H. Investigation, K.R.J.-W. and T.F.G. Formal analyses, Y.M.E., M.P., T.F.G., M.G.H., and L.J.W. Data Curation: Y.M.E., M.P., M.G.H., and L.J.W. Writing – Original Draft, Y.M.E., M.P., M.G.H., and T.F.G. Writing – Review and Editing, Y.M.E., M.P., C.P.M., T.F.G., G.S.D., J.F.M., and L.P. Visualization: M.P. and M.G.H.

DECLARATION OF INTERESTS

B.O. has consulted for Biogen, MediciNova, Mitsubishi, Amylyx, and Tsumura. K.A.N. has performed consulting for Alector, AI Therapeutics, Biogen, MT Pharma, Avanir Pharmaceuticals, and Biohaven. J.B.H. is the CLIA director for the Lung Bioengineering facility at Mayo Clinic Florida campus, a non-Mayo corporation. Z.K.W. also serves as PI or Co-PI on Biohaven Pharmaceuticals, Inc. (BHV4157-206 and BHV3241-301), Neuraly, Inc. (NLY01-PD-1), and Vigil Neuroscience, Inc. (VGL101-01.001 and VGL101-01.002) grants; as well as a Co-PI of the Mayo Clinic APDA Center for Advanced Research and as an external advisory board member for the Vigil Neuroscience, Inc. L.P. is a consultant for Expansion Therapeutics. The other authors declare that they have no competing interests.

Received: June 23, 2022
Revised: September 13, 2022
Accepted: September 30, 2022
Published: November 18, 2022

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human plasma	Laboratory of Leonard Petrucelli, Mayo Clinic Florida	
Critical commercial assays		
NF-light	Quanterix	Cat#103186
Other		
Simoa HD-1 Analyzer instrument	Quanterix	

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Leonard Petrucelli (petrucelli.leonard@mayo.edu).

Materials availability

This study did not generate unique reagents.

Data and code availability

- All data associated with this study are present in the paper or the supplemental information.
- Requests for de-identified data should be addressed to the lead investigator and will made available through a data transfer agreement.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
- This paper does not report original code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Protocol approvals and patient consents

Study samples were collected through the Neurological Disease Biorepository and Biomarker Initiative at Mayo Clinic's campus in Jacksonville, Florida. To identify a cohort of individuals with COVID-19, we queried the Mayo Clinic Neurological, Vascular and Neurovascular Events With SARS-CoV-2 Study (MC NEWS; Institutional Review Board (IRB) #20-003457). MC NEWS included patients across the three major campuses of Mayo Clinic and the Mayo Clinic Health System, with hospitals in Arizona, Florida, Minnesota, and Wisconsin. However, for this study only Florida campus patient samples were included. Plasma samples from patients with COVID-19 and healthy control individuals were obtained under IRB approval through the following protocols: "Investigating biomarkers, disease mechanisms and treatments for spinocerebellar ataxia and nucleotide repeat diseases", IRB#17-006033; "Investigating the Genetic and Phenotypic Presentation of Spinocerebellar Ataxia and Nucleotide Repeat Diseases", IRB#16-009414; "Biospecimen Biorepository for the Study of ALS, ALS-FTD and Similar Neurodegenerative Disorders", IRB#13-004314; "Pilot Evaluation of Neurofilament Heavy Form (NF-H) as a Potential Biomarker of Axonal Loss in Amyotrophic Lateral Sclerosis (ALS)", IRB#10-003592; "Clinical & Genetic Studies in ALS, Suspected ALS, and Other Neurodegenerative Motor Neuron Disorders", IRB#07-005711; "Biospecimen Collection to Investigate the Causes of ALS", IRB# 15-001187; "The DIALS (Dominant Inherited ALS) Network", IRB# 2017P000485; and "COVID-19 cytokine storm project", IRB#20-003661.

Human subject characteristics

Human subject characteristics are provided in Tables 1, S3, and S5, and include data on age, sex, race, BMI, CKD, vaccination status, length of time from admission to blood draw, and Charlson comorbidity index

scores. The Charlson comorbidity index score is a validated clinical score that predicts a patient's risk of mortality based on the number conditions suffered by a given patient (Charlson et al., 1987, 1994). A total of 880 patients with COVID-19 for whom plasma was collected within 5 days of admission to Mayo Clinic Florida hospital from June 2020 to October 2021 were included in the study. Eleven of the 880 patients were part of our previous study cohort (Prudencio et al., 2021) but only in two of them were NFL concentrations measured at the same time point. Healthy control samples (N = 55) were collected prior the COVID-19 pandemic (February 2011 to July 2019) and were composed of healthy individuals from the general population, spouses, friends and/or caregivers of patients with genetically caused diseases and who lacked disease-associated mutations. Demographics and clinical information for patients with COVID-19 were abstracted using shared electronic medical record (Epic systems Corporation, Verona, WI). The following patient outcomes were collected: need for mechanical ventilation (intubation), ICU admission, LOS, mRS scores at discharge, vital status on follow-up and death. The mRS score indicates and individual's degree of disability (Nunn et al., 2016). It consists of a seven-level ordered categorical scale defined as: 0 = fully independent; no symptoms; 1 = no significant disability despite symptoms, able to perform all usual duties and activities; 2 = slight disability, unable to perform all previous activities but able to look after own affairs without assistance; 3 = moderate disability, requiring some help but able to walk without assistance; 4 = moderately severe disability, unable to walk without assistance and unable to attend to own bodily needs without assistance; 5 = severe disability, bedridden, incontinent and requiring constant nursing care and attention; 6 = death) (Dromerick et al., 2003).

METHOD DETAILS

Plasma neurofilament light concentration determination

Plasma NFL concentrations were measured as previously described (Prudencio et al., 2021), using the Simoa HD-X analyzer (NF-Light digital immunoassay, Quanterix, Cat #103186) and following manufacturer's instructions. In short, plasma samples were thawed on ice and cleared by centrifugation (10,000 xg for 5 minutes) and transferred to 96-well plates. Samples were run in a blinded fashion, and in duplicate using a 4x instrument dilution. To monitor potential variability among assays four reference samples of known differing NFL concentrations were included in every run, along with two quality controls provided by the manufacturer. NFL concentrations were interpolated from the calibration curve. Samples with NFL concentrations that exceeded the range of the assay were retested using an appropriate at-bench dilution in addition to the 4x instrument dilution.

The mean %CV of duplicate NFL measurements was 3.94%. Across seven runs, the mean concentration of the first reference sample was 4.09 pg/mL and the inter-assay %CV was 12.4%, the mean concentration of the second reference sample was 8.48 pg/mL and the inter-assay %CV was 8.7%, the mean concentration of the third reference sample was 15.79 pg/mL and the inter-assay %CV was 9.17%, the mean concentration of the fourth reference sample was 187.6 pg/mL and the inter-assay %CV was 10.8%.

QUANTIFICATION AND STATISTICAL ANALYSES

Continuous variables were summarized with the sample median and range. Categorical variables were summarized with number and percentage of patients. Plasma NFL was examined on the base-2 logarithm scale in all regression analyses owing to its skewed distribution. Covariates that were adjusted for in multivariable regression models were pre-defined. The number of individuals in each group for a given statistical analyses is reported in the corresponding table/figure. Multiple testing was adjusted for separately for each group of similar statistical test. All statistical tests were two-sided and were performed using SAS (version 9.4; SAS Institute, Inc., Cary, North Carolina).

Comparisons of plasma NFL concentration between patients with COVID-19 and controls

To evaluate the differences in plasma NFL concentration between healthy controls and patients with COVID-19, we used a stratified van Elteren Wilcoxon rank sum test (van Elteren, 1960), where the test was stratified by both age as a four-level categorical variable (based on sample quartiles) and sex (Figure 1). $p < 0.05$ was considered statistically significant.

Associations of plasma NFL in patients with COVID-19 with clinical outcomes

We assessed associations between plasma NFL and the following clinical outcomes in COVID-19 patients: intubation, ICU admission, LOS, and mRS at discharge (Table 2). Given that these clinical outcomes

occurred either prior to measurement of some NFL values (intubation or ICU admission) or at a similar time point when NFL was measured (intubation, ICU admission, LOS, and mRS at discharge), performing an analysis that examines the ability of NFL to predict these outcome measures is not possible given our data. For this reason, we assessed associations between these four variables and NFL when considering NFL as the dependent variable, in order to avoid any implications regarding the predictive utility of NFL for these four outcomes. Associations between plasma NFL and outcomes were made using linear regression models. Regression coefficients and 95% CIs were estimated, and are interpreted as the change in mean NFL concentration (on the base-2 logarithm scale) corresponding to presence of the given outcome (intubation or ICU admission), to each doubling of length of hospital stay, and to each 1-unit increase in mRS at discharge.

Unadjusted models and multivariable models that were adjusted for age, sex, BMI, CKD, race, vaccination status (none, partial, full), and Charlson comorbidity index scores were evaluated. We did not initially adjust for length of time from admission to blood draw for NFL measurement in multivariable models since this variable can also possibly be considered as an outcome measure (as a longer time from admission to blood draw indicates a longer hospitalization and likely worse outcomes) and therefore could be on the causal pathway between NFL and outcomes (Schisterman et al., 2009). However, in a sensitivity analysis, we did also additionally adjust for length of time from admission to blood draw in our multivariable models (Tables S2, S4, and S6).

We utilized a Holm step-down correction for multiple testing (Holm, 1979) in the multivariable analysis separately for each family of similar statistical tests. After applying this adjustment, p-values <0.05 (given that the lowest three p-values were lower than the 0.0125, 0.0167, and 0.025 significance thresholds of the step-down correction) were considered as significant when assessing associations between plasma NFL and outcomes.

Comparisons of patient characteristics between white and black/African American, and between unvaccinated and fully vaccinated

Comparisons of patient characteristics between white and black/African American patients, and also between unvaccinated and fully vaccinated patients, were made using a Wilcoxon rank sum test (continuous and ordinal variables) or Fisher's exact test (categorical variables). $p < 0.05$ were considered statistically significant.

Comparisons of clinical outcomes and plasma NFL concentrations between white and black/African Americans, and between unvaccinated and fully vaccinated patients with COVID-19

Comparisons of outcomes and plasma NFL concentrations (all as dependent variables) between white and black/African Americans patients and between unvaccinated and fully vaccinated patients were made using binary logistic regression models (intubation and ICU admission), negative binomial regression models (length of hospital stay), proportional odds logistic regression models (mRS at discharge), and linear regression models (plasma NFL). All estimates are given in comparison to a reference group of white patients or fully vaccinated patients. For intubation and ICU admission, odds ratios (ORs) and 95% CIs are interpreted as the multiplicative increase in the odds of the given outcome. For length of hospital stay, the multiplicative effect on the mean and 95% CI are interpreted as the multiplicative increase in mean length of hospital stay. For mRS at discharge, the OR and 95% CI are interpreted as the multiplicative increase in the odds of a higher mRS at discharge. For plasma NFL, the regression coefficient is interpreted as the difference in mean plasma NFL level (on the base 2 logarithm scale). Unadjusted analysis and multivariable models were examined, except for models (even unadjusted models) involving the outcome of plasma NFL being additionally adjusted for time from admission to blood draw. Additionally, in sensitivity analysis we also adjusted for length of time from admission to blood draw in our multivariable models involving clinical outcomes (Tables S2, S4, and S6).

We utilized a Holm step-down correction for multiple testing [3] in the multivariable analysis separately for each family of similar statistical tests. After applying this adjustment, p-values <0.010 were considered as significant when comparing outcomes and NFL concentration between white and black/African American patients, and p-values <0.0125 were considered significant when comparing outcomes and NFL concentration between unvaccinated and fully vaccinated patients.