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Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19

[1,2]Nelly Kanberg, M.D.; [3,4,5]Nicholas J. Ashton, Ph.D.; [1,2]Lars-Magnus Andersson, M.D., Ph.D.; [1,2]Aylin Yilmaz, M.D., Ph.D.; [1]Magnus Lindh, M.D., Ph.D.; [6]Staffan Nilsson, Ph.D.; [10]Richard W Price, M.D., Ph.D.; [3,7]Kaj Blennow, M.D., Ph.D.; [3,7,8,9]Henrik Zetterberg, M.D., Ph.D., [1,2]Magnus Gisslén, M.D., Ph.D.

[1] Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[2] Region Västra Götaland, Sahlgrenska University Hospital, Department of Infectious Diseases, Gothenburg, Sweden
[3] Department of Psychiatry and Neurochemistry, Institute of Neuroscience & Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
[4] Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden
[5] King's College London, Institute of Psychiatry, Psychology & Neuroscience, Maurice Wohl Clinical Neuroscience Institute, London, UK; 4NIHR Biomedical Research Centre for Mental Health & Biomedical Research Unit for Dementia at South London & Maudsley NHS Foundation, London, UK
[6] Department of Mathematical Sciences, Chalmers University of Technology, Gothenburg, Sweden
[7] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
[8] Department of Neurodegenerative Disease, UCL Institute of Neurology, London, United Kingdom
[9] UK Dementia Research Institute at UCL, London, United Kingdom
[10] Department of Neurology, University of California San Francisco, San Francisco, USA.

Corresponding authors: Magnus Gisslén, M.D., Ph.D., Email: magnus.gisslen@infect.gu.se

Statistical Analysis:
1. **Corresponding author** (affiliations above).
2. **Staffan Nilsson**
   Reader, Applied Mathematics and Statistics
   Department of Mathematical Sciences
   Chalmers University of Technology, Gothenburg, Sweden

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Abbreviations used in this paper: CNS, central nervous system; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ACE-2, angiotensin converting enzyme 2; NfL, neurofilament light protein; GFAp, glial fibrillary acidic protein

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Abstract

Objective

To test the hypothesis that COVID-19 has an impact on the CNS by measuring plasma biomarkers of CNS injury.

Methods

We recruited 47 patients with mild (n=20), moderate (n=9) or severe (n=18) COVID-19 and measured two plasma biomarkers of CNS injury by Single molecule array (Simoa): neurofilament light chain protein (NfL) (a marker of intra-axonal neuronal injury) and glial fibrillary acidic protein (GFAp) (a marker of astrocytic activation/injury) in samples collected at presentation and again in a subset after a mean of 11.4 days. Cross-sectional results were compared with 33 age-matched controls derived from an independent cohort.

Results

The patients with severe COVID-19 had higher plasma concentrations of GFAp (p=0.001) and NfL (p<0.001) than controls, while GFAp was also increased in patients with moderate disease (p=0.03). In severe patients an early peak in plasma GFAp decreased upon follow-up (p<0.01) while NfL showed a sustained increase from first to last follow-up (p<0.01), perhaps reflecting a sequence of early astrocytic response and more delayed axonal injury.

Conclusion

We show neurochemical evidence of neuronal injury and glial activation in patients with moderate and severe COVID-19. Further studies are needed to clarify the frequency and nature of COVID-19-related CNS damage, and its relation to both clinically-defined CNS events such as hypoxic and ischemic events and to mechanisms more closely linked to systemic SARS-CoV-2 infection and

consequent immune activation, and also to evaluate the clinical utility of monitoring plasma NfL and GFAp in management of this group of patients.

Introduction

Early in the SARS-CoV-2 outbreak, a case series from Wuhan, China reported CNS involvement in 36% of patients hospitalized for severe COVID-19 infection (1). CNS involvement has been previously described in hospitalized patients infected with SARS-CoV during the 2003-2004 SARS epidemic (2). Further, SARS‐CoV was isolated from brain tissue with edema and neuronal degeneration at autopsy, confirming viral infection of the neurons (2, 3). Given the taxonomical similarity between SARS-CoV and SARS-CoV-2, it is plausible that patients suffering from COVID-19 might also exhibit CNS damage related to the infecting coronavirus. It remains unclear to what extent SARS-CoV-2 is able to infect the CNS and if so, how the virus reaches the brain, but two possible theories have emerged: spread across the cribriform plate of the ethmoid bone in proximity to the olfactory bulb in patients at the early stage of the disease resulting in the relatively common loss of sense of smell (4), or a later occurring haematogenous spread on the setting of accompanied hypoxia, respiratory, and metabolic acidosis (5). Direct CNS infection by SARS-CoV has also been shown in mice (6), but whether the SARS-CoV-2 infects the brain of humans remains unknown.

To assess the broad impact of COVID-19 on CNS and test the hypothesis that COVID-19 is accompanied by underappreciated CNS injury, we analysed two plasma biomarkers for CNS injury (glial fibrillary acidic protein [GFAp] and neurofilament light chain [NfL]) in COVID-19 patients and matched controls. GFAp is an intermediate filament, highly expressed in astrocytes, and serves as a marker of astrocytic activation/injury (7). NfL is an intra-axonal structural protein and a biomarker of neuronal injury (8). While extensively studied in CSF, recent sensitive methods have shown that plasma measurement of both biomarkers reliably detect CNS injury and correlate with clinical outcomes in a range of conditions (8). The aim of this study was to examine the extent of CNS

involvement in patients with COVID-19 as indicated by these two established biomarkers of CNS disease diagnosis and progression.

Methods

Study population

Forty-seven patients with confirmed COVID-19 were divided into 3 groups related to systemic disease severity: 20 patients with mild (*i.e.*, not requiring hospitalization) 9 with moderate (hospitalized and requiring oxygen supplementation), and 18 with severe disease (admitted to intensive care unit [ICU] and placed on mechanical ventilation [n=17] or not considered a candidate for ICU treatment and with fatal outcome [n=1]). The biomarker findings were compared to those of an age-matched controls (n=33) that were initially recruited as cognitively unimpaired controls for an observational study on risk factors for neurodegeneration. None of them had psychiatric or neurological comorbidity and any magnetic resonance imaging (MRI) abnormalities were set as an exclusion criterion.

Blood samples were collected from a subgroup of patients at a mean (SD) of 13.0 (7.37) days after onset of symptoms (16.0 (9.85) days in mild, 11.6 (2.19) in moderate, and 10.4 (4.35) days in severe disease). In 31 of the patients, follow-up specimens were collected up to a mean (SD) of 11.4 (5.06) days after the first sampling. Follow-up samples on patients with severe COVID-19 were collected during ongoing ICU hospitalization.

Viral diagnostic methods

The diagnosis of SARS-CoV-2 infection was confirmed using real-time polymerase chain reaction (rtPCR) analysis of nasal and throat swab specimens. Nucleic acid was extracted from clinical samples in a MagNA Pure 96 instrument using the Total Nucleic Acid isolation kit (Roche). rtPCR

targeting the RdRP region was performed in a QuantStudio 6 instrument (Applied Biosystems, Foster City, CA) using the probe described by Corman et al. and the primers RdRP_Fi, GTCATGTGTGGCGGTTCACT and RdRP_Ri, CAACACTATTAGCATAAGCAGTTGT (9).

Biomarker analyses

All plasma GFAp and NfL measurements were performed in the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital by board-certified laboratory technicians blind to clinical data using commercially available single molecule array (Simoa) assays on an HD-X Analyzer (Human Neurology 4 - Plex A assay (N4PA advantage kit, 102153), as described by the manufacturer (Quanterix, Billerica, MA). A single batch of reagents was used; intra-assay coefficients of variation were below 10% for all analytes. Because in acute brain injury plasma GFAp increases rapidly and has a short half-life of 24-48 hours while plasma NfL increases later and remains elevated for > 10 days (10), for patients with multiple sampling available we used the first sample for GFAp, and the last for NfL in cross-sectional comparisons between groups.

Statistical analyses

All data are reported as mean and standard deviation, unless otherwise indicated. Associations were measured with Pearson correlation. Estimated geometric means at age 70 were compared for the three COVID-19 groups with controls by analysing log10 plasma levels with ANCOVA adjusting for age and, including interactions between age and group. Changes in log concentrations from first to last measure were analysed with paired t-test. A p value < 0.05 was considered significant. Analyses result and graphs were generated using SPSS statistics (IBM SPSS version 25) or Prism (GraphPad Software version 8.00, La Jolla, California, USA).

Standard protocol approvals, registrations, and patient consents

This study has been approved by the Swedish Ethical Review Authority (2020-01771). All participants provided informed consent, in those with severe COVID-19, this was obtained before they were placed on mechanical ventilation and were deemed fully capable of understanding the nature of the study and their part in it.

Data availability

Researchers can apply for access to anonymized data from the present study for well-defined research questions that are in line with the overall research agenda for the cohort. Please contact the corresponding author.

Results

Demographics

All patients had a confirmed SARS-CoV-2 infection. Those in the mild group were generally younger and otherwise healthy, while the moderate and severe patients were mostly men, older and had more comorbidities (table 1). The control group consisted of 16 women and 17 men with a median (IQR) age of 67.0 (42.3-77.8) years.

Four patients had symptoms of confusion before admission to the ICU, and one had a single episode of seizure before transfer to the ICU with no signs of epileptic activity at EEG performed the day after. CT scans were normal in two of the three cases scanned; the third had signs of small-vessel disease. MRI scans were not performed due to restrictions imposed by the protection of hospital workers and other patients in place at the time. No additional neurological abnormalities were documented.

Biomarkers

Both plasma GFAp ($r = 0.62$, $p < 0.001$) and NfL ($r = 0.62$, $p < 0.001$) were correlated with age, both for patients with COVID-19 and controls, figure 1 A-B. Concentrations of GFAp and NfL in the different subgroups can be found in table 2. Patients with severe COVID-19 had significantly higher plasma concentrations of GFAp ($p = 0.001$) and NfL ($p < 0.001$) than controls, and GFAp was increased also in patients with moderate disease ($p = 0.03$), figure 1 A-B. Patients with severe COVID-19 had 78 % (CI95 27-150 %) higher plasma concentrations of GFAp ($p = 0.001$) and 208 % (CI95 120-329 %) higher NfL ($p < 0.001$) than controls when comparing the estimated geometrical means at age 70. Plasma GFAp was 56 % (CI95 4-133 %) higher in patients with moderate disease compared to controls. A correlation was found between plasma GFAp and NfL ($r = 0.580$, $p < 0.001$), Figure 1C.

Neither plasma GFAp, nor NfL, changed significantly from the initial to the last follow-up in patients with mild or moderate disease, figures 2A and C. In contrast, in the severe group, plasma GFAp decreased from in median 215 (IQR 106-281) pg/mL at the initial to 103 (60-225) pg/mL at the last sampling ($p = 0.004$), figure 2B, and plasma NfL concentrations increased from in median 20 (11-24) pg/mL in the first to 32 (16-60) pg/mL in last specimen ($p = 0.002$), Fig 2D.

Plasma NfL concentration correlated inversely with the blood lymphocyte count, a negative prognostic factor (11) ($r = -0.37$, $p = 0.047$); there was no significant correlation with C-reactive protein (CRP) concentrations (data not shown).

Discussion

We have examined two blood-based biomarkers for CNS injury in patients with COVID-19. NfL and GFAp have historically proved useful measures of CNS injury when assessed in CSF, but sampling of this fluid is challenging in the clinical COVID-19 setting. In contrast, measurement of these markers in the plasma is convenient and provides a practical method of assessing the effect of COVID-19 on

the CNS. This approach follows on extensive validation of their ability to detect CNS injury in several conditions, including neurodegenerative disorders, multiple sclerosis, HIV and cardiac arrest (12-15).

The results of this study indicate that astrocytic activation/injury (GFAp measurements) may be a common feature in moderate and severe stages of COVID-19, while neuronal injury (NfL) occurs later in the disease process and mainly in patients with severe disease. One may hypothesize that astrocytic activation/injury is a first response to CNS insult and that plasma NfL increase reflects a progression to neuronal injury in severe cases.

The pathogenesis of these CNS effects of COVID-19 is not known, although direct invasion of the virus may be unlikely. The entry of SARS-CoV-2 into human host cells is mediated mainly by the cellular receptor angiotensin‐converting enzyme 2 (ACE2), which is expressed at very low levels in the CNS under normal conditions (3). CNS hypoxia due to respiratory failure caused by COVID-19, thrombotic microangiopathy, or an indirect effect of the vigorous inflammatory response with extensive cytokine activation that is commonly found in severe COVID-19, are more probable explanations, although further study is needed to examine these factors.

Our study had several limitations. Firstly, it included a limited number of participants. Secondly, due to restrictions imposed to isolate and protect personnel and equipment, and since all our severely ill patients were admitted to the ICU and on mechanical ventilators, a thorough neurological and cognitive evaluation was not done, and long-term follow ups were limited. Also, a potential impact of confounding factors, such as vascular risk factors, has not been possible to fully account for. None of the controls had any inadequately treated condition but data on treated comorbidities are lacking.

In conclusion, our results show that plasma biomarkers of CNS damage are increased in patients with COVID-19 and associated with disease severity. Further studies are needed to clarify the nature of CNS injury in this setting and to further evaluate the utility of these biomarkers in COVID-19.

Appendix 1 Authors

Name	Location	Contribution
Nelly Kanberg, M.D.	Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden	Manuscript writing and revision, data acquisition and interpretation
Nicholas J Ashton, Ph.D.	Department of Psychiatry and Neurochemistry, Institute of Neuroscience & Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden	Data acquisition and manuscript revision
L-M Andersson, M.D., Ph.D.	Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden	Patient recruitment and manuscript revision
Aylin Yilmaz, M.D., Ph.D.	Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden	Patient recruitment and manuscript revision
Magnus Lindh,	Department of Infectious	Data acquisition and manuscript

M.D., Ph.D.	Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden	revision
Staffan Nilsson, Ph.D.	Department of Mathematical Sciences, Chalmers University of Technology, Gothenburg, Sweden	Statistical advice and analysis
Richard W Price, M.D., Ph.D.	Department of Neurology, University of California San Francisco, San Francisco, USA.	Data interpretation and manuscript revision
Kaj Blennow, M.D., Ph.D.	Department of Psychiatry and Neurochemistry, Institute of Neuroscience & Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden	Data acquisition and interpretation & manuscript revision
Henrik Zetterberg, M.D., Ph.D.	Department of Psychiatry and Neurochemistry, Institute of Neuroscience & Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden	Study concept and design, data analysis and interpretation, manuscript revision
Magnus Gisslén, M.D., Ph.D.	Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University	Study concept and design, patient recruitment, data analysis and interpretation,

	of Gothenburg, Gothenburg, Sweden	manuscript revision, study supervision

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Figure legends

Figure 1: Plasma concentrations of blood-based central nervous system biomarkers in patients with mild, moderate, and severe COVID-19 compared to healthy controls.

Log_{10} plasma levels were analysed with ANCOVA, including interactions between age and group (A-B). Estimated geometric means at age 70 for the three COVID-19 groups were compared with controls.

(A) Age and plasma GFAp were significantly correlated. Plasma levels of GFAp were significantly increased in moderate and severe COVID-19 groups as compared to controls ($p = 0.03$ and $p = 0.001$, respectively).

(B) Age and plasma NfL were significantly correlated. Plasma levels of NfL were 3.1 times higher in patients with severe COVID-19 as compared to controls ($p < 0.001$).

(C) Correlation between log_{10} values of plasma GFAp and NfL in patients with COVID-19.

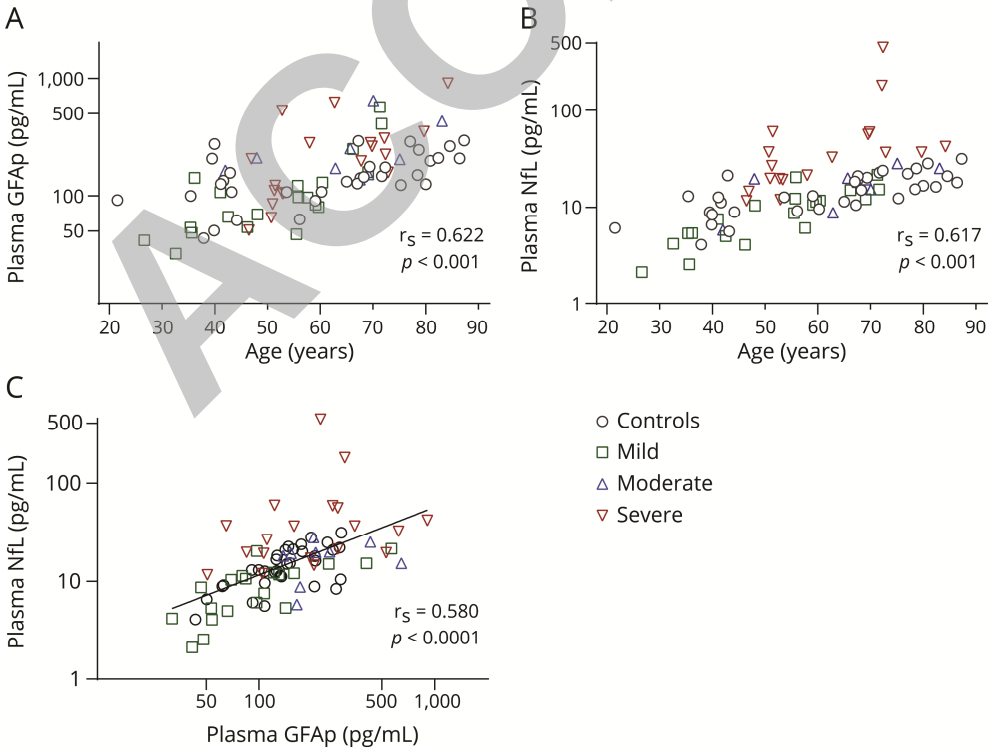

Figure 2: Plasma concentrations of GFAp and NfL in relation to onset of COVID-19 symptoms

Plasma GFAp and NfL concentrations in patients with mild (green squares), moderate (blue triangles), and severe (red triangles) COVID-19. Lines connect multiple sampling in individual patients. No significant changes from initial to last follow-up were found in mild or moderate disease (A and C). In contrast, a significant decrease in plasma GFAp (p = 0.004) and increase in plasma NfL (p = 0.002) were found in severe COVID-19 (B and D).

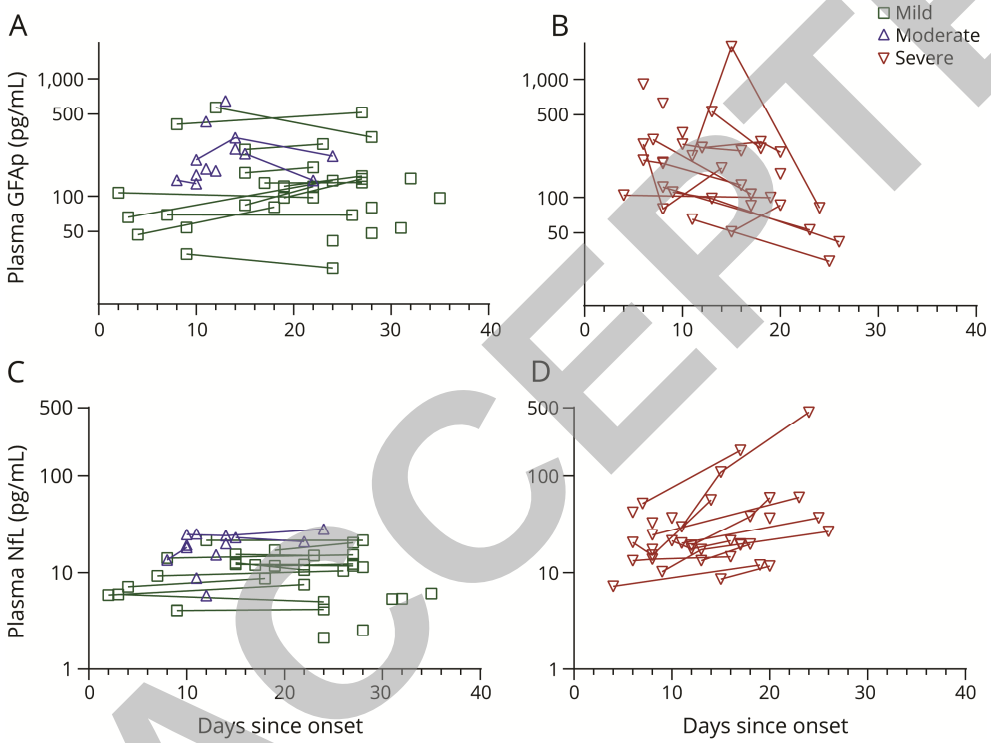

Table 1. Patient Characteristics

	Total	**Mild**	**Moderate**	**Severe**
	(n=47)	(n=20)	(n=9)	(n=18)
Demographic characteristics				
Age, median (IQR), years	57.8 (48.0-69.5)	55.6 (37.4-60.2)	67.5 (55.4-72.6)	58.0 (51.3-72.2)
Sex				
Female	15 (32%)	10 (50%)	3 (33%)	2 (11%)
Male	32 (68%)	10 (50%)	6 (67%)	16 (89%)
Comorbidities				
Any	18 (38%)	1 (5%)	5 (56%)	12 (67%)
Hypertension	12 (26%)	0	2 (22%)	10 (56%)
Obesity	3 (6%)	0	1 (11%)	2 (11%)
Diabetes	9 (19%)	0	2 (22%)	7 (39%)
Coronary heart disease	7 (15%)	1 (5%)	2 (22%)	4 (22%)
Malignancy	1 (2%)	0	0	1 (6%)

Table 2. Median (IQR) plasma concentrations of GFAp and NfL in different subgroups of COVID-19 and controls

	Mild	**Moderate**	**Severe**	**Controls**
	(n=20)	(n=9)	(n=18)	(n=33)
Plasma GFAp concentrations, median (IQR) pg/mL	90.5 (53.5-139)	204 (158-341)	206 (106-308)	141 (108-207)
Plasma NfL concentrations, median (IQR) pg/mL	9.5 (5.1-12.2)	19.3 (12.1-22.6)	32.7 (19.3-56.3)	13.1 (9.4-21.0)

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