Blood neurofilament light chain and total tau levels at admission predict death in COVID-19 patients

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Abstract

Background and aims Patients infected with SARS-CoV-2 range from asymptomatic, to mild, moderate or severe disease evolution including fatal outcome. Thus, early predictors of clinical outcome are highly needed. We investigated markers of neural tissue damage as a possible early sign of multisystem involvement to assess their clinical prognostic value on survival or transfer to intensive care unit (ICU).

Methods We collected blood from 104 patients infected with SARS-CoV-2 the day of admission to the emergency room and measured blood neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), and total tau protein levels.

Results We found that NfL, GFAP, and tau were significantly increased in patients with fatal outcome, while NfL and UCH-L1 in those needing ICU transfer. ROC and Kaplan–Meier curves indicated that total tau levels at admission accurately predict mortality.

Conclusions Blood neural markers may provide additional prognostic value to conventional biomarkers used to predict COVID-19 outcome.

Keywords Neurofilament light chain · GFAP · UCH-L1 · Tau · SARS-CoV-2 · COVID-19

Introduction

Direct invasion of the central nervous system (CNS) by SARS-CoV-2 is a controversial issue, with contradictory findings generating a lively debate in current literature [1–4]. Acute respiratory distress syndrome (ARDS), however, is associated to nervous system damage and neurological sequelae also in the absence of a clinically apparent CNS infection [5, 6]. The Coronavirus disease 2019 (COVID-19) is no exception, and neurological and psychiatric consequences of the disease have been described in large patients series [7, 8] and are associated to MRI and EEG changes [7, 8]. COVID-19 is highly heterogeneous in terms of clinical outcome, ranging from no or mild symptoms to the need of life support in intensive care units (ICU) or to fatal outcome. The search for predictors able at patient admission to direct clinical choices would be beneficial to set priorities and optimize interventions in circumstances requiring highly coordinated clinical efforts to avoid hospitals, and especially ICU, overcrowding. CNS biomarkers might reflect early
multiorgan involvement of COVID-19, typical of patients with more severe disease evolution. Neurofilament light chain (NfL) is a cytoskeletal intermediate filament protein of central and peripheral neurons [9], and its detection in the blood has been now validated as a nervous system damage biomarker in a variety of neurological diseases [10]. Glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) are astrocytic- and neuronal-specific proteins, respectively, recently recognized by FDA as CNS damage biomarkers in traumatic brain injury [11, 12]. Tau protein is mostly expressed by neurons, implicated in the pathogenesis of tauopathies, and employed as biomarker of brain damage not only in neurodegenerative diseases [13]. We measured NfL, GFAP, UCH-L1, and total tau in a cohort of COVID-19 patients at admission at the Emergency Department (ED), and evaluated their prognostic value on the clinical outcome.

Methods

Patients

This retrospective cohort investigation is part of the COVID-BioB study, a comprehensive observational study conducted at the San Raffaele University Hospital, a tertiary health-care center in Milan, Italy. All patients aged ≥ 18 years admitted to San Raffaele University Hospital for COVID-19 from 25 February 2020 were included in the COVID-BioB study [14]. COVID-19 was diagnosed based on a positive SARS-CoV-2 real-time reverse-transcriptase polymerase chain reaction (RT-PCR) from a nasopharyngeal swab and clinical and/or radiologic findings of COVID-19 pneumonia. In line with the COVID-BioB protocol, blood samples from all patients were collected at ED arrival and stored in a dedicated biobank set up specifically for the current pandemic [15]. Detailed demographic, laboratory, and clinical data of patients were recorded in an electronic case record form (eCRF). Specifically, laboratory data included conventional markers of COVID-19 severity such as the absolute lymphocyte and neutrophil counts, neutrophil to lymphocyte ratio (NLR), lactate dehydrogenase (LDH), and C-reactive protein (CRP) at ED presentation. The ratio of arterial oxygen partial pressure (PaO₂) in mmHg to fractional inspired oxygen (FiO₂) expressed as a fraction (PaO₂/FiO₂) at hospital admission was also extracted for all patients as a marker of the degree of hypoxia. One hundred four patients evaluated at our Institution during the first wave of the pandemic between March 18th and May 5th, 2020 were included in the present analysis. Median (interquartile range, IQR) time from hospital admission to venepuncture was 1 (0–2) days. The COVID-BioB study protocol was approved by the Ethics Committee (protocol no. 34/int/2020) and registered on ClinicalTrials.gov (NCT04318366). It is in accordance with the Declaration of Helsinki and all patients signed informed consent.

Sample processing and biomarker measurement

Plasma-EDTA was obtained from venous blood, immediately frozen and maintained at − 80 °C until subsequent analyses. Plasma was inactivated using tri-(n-butyl) phosphate and Triton X-100 (Sigma) (0.3% and 1%, respectively) for 2 h [16]. NfL, GFAP, UCH-L1, and total tau, were measured with Simoa Human Neurology 4-Plex B assay (N4PB) on a Quanterix SIMOA HD-1 platform according to manufacturer instructions [17].

Statistical analysis

Categorical variables were expressed as absolute frequencies (percentage), and continuous variables as median (IQR). Comparisons in continuous and categorical variables between independent groups were performed using, respectively, Mann–Whitney test and chi-squared test or Fisher test, as appropriate. Spearman’s correlation coefficient was employed to investigate pairwise correlations between continuous variables. The area under the curve (AUC) of the receiving operator characteristic (ROC) curve of the predicted probabilities was used to estimate the accuracy of models including the studied biomarkers in predicting fatal outcome. Kaplan–Meier curves were used for survival analysis and the log-rank test was used to assess differences in survival between groups. Statistical analyses were performed using R statistical package (version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria), with a two-sided significance level set at p < 0.05.

Results

One hundred and four patients were included in the present study. Patient and disease characteristics at hospital admission as well as clinical outcomes of the cohort are reported in Table 1. Overall, 34 (33%) patients required transfer to ICU and 22 (21%) patients died of COVID-19 or related complications. Seventy-seven (74%) patients were naïve from any COVID-19-specific treatment at time of blood draw. The remaining patients had started one or more therapeutic agents among hydroxychloroquine, steroids, lopinavir/ritonavir, and low molecular weight heparin for a median time of 1 (0–2) days.
Biomarkers of CNS damage correlate with conventional markers of COVID-19 severity

We correlated blood biomarkers of CNS damage with conventional biomarkers routinely used in COVID-19 prognostics such as PaO₂/FiO₂ (Horowitz index), NLR, absolute lymphocyte and neutrophil counts, concentration of CRP and LDH, and creatinine (Supplementary Fig. 1). We found that levels of NfL significantly and strongly correlated with all conventional biomarkers and a similar trend was observed for tau, GFAP, and UCH-L1 although with lower correlation coefficients.

Neural biomarkers in the blood at admission are increased in patients with fatal COVID-19

Patients with fatal COVID-19 displayed at ED admission significantly increased blood levels of NfL [36.1 pg/ml (16.7–49.5) vs. 17.1 (11.4–28.4) in survivors; Fig. 1A], GFAP [314.7 pg/ml (160.9–445.1) vs. 175.4 (113.5–325.1) in survivors; Fig. 1B], and tau [2.0 pg/ml (0.6–4.7) vs. 0.4 (0.1–1.2) in survivors; Fig. 1D], but not UCH-L1 [29.7 pg/ml (20–40.1) vs. 18.4 (13.8–30) in survivors; Fig. 1C].

When considering patients needing transfer to ICU (including those with fatal outcome), only NfL [26.3 pg/ml (15.9–44) vs. 16.9 (11.2–29.2) in survivors; Fig. 1E] and UCH-L1 [27.6 pg/ml (17.4–40.1) vs. 17.3 (13.5–28.5) in survivors; Fig. 1G] levels were significantly increased, while GFAP (Fig. 1F) and tau (Fig. 1H) were not different.

Neural blood biomarkers measured at admission are predictive of fatal COVID-19 evolution

To answer the question whether CNS tissue damage biomarkers are prognostic of COVID-19 outcome, we performed ROC curves analyses. We found that NfL, GFAP, UCH-L1, and tau all had a significant predictive value (Fig. 2A–D), the best predictor being tau with an AUC of 0.7722. We next verified whether a combination of neural markers increases prediction accuracy, but both the combination of all four markers (Fig. 2E, AUC = 0.7639) and the one excluding the worst predictor, UCH-L1, (Fig. 2F, AUC = 0.7622) did not yield a better performance. To verify the ability of tau blood levels to predict fatal outcome we performed survival analysis dividing the cohort based on whether tau blood levels at admission were above or below the median value of 0.5 pg/ml. Indeed, survival analysis showed that mortality significantly increased in patients with higher tau levels at admission (Fig. 3).

Discussion

The search for predictors of COVID-19 evolution has yielded a number of prognostic markers for COVID-19-related mortality such as older age, coronary artery disease, cancer, low lymphocyte count, and high radiographic assessment of lung edema (RALE) score [14], including laboratory markers for organ dysfunction [18]. Previous studies have investigated also CNS biomarkers. Some investigators have focused on cerebrospinal fluid alterations with the aim of helping identify patients with neurological complications, showing that, as expected, CNS tissue damage biomarkers

Table 1 Demographic, clinical, and serological features of COVID-19 patients

|                  | n = 104 |
|------------------|---------|
| Age (years)      | 57.8 (48.5–67) |
| Female sex       | 40 (38.5) |
| Ethnicity        |          |
| Caucasian        | 84 (80.8) |
| Hispanic         | 15 (14.4) |
| Asian            | 3 (2.9)  |
| African–American | 2 (1.9)  |
| Comorbidities    |          |
| HTN              | 38 (36.5) |
| CAD              | 8 (7.7)  |
| DM               | 21 (20.2) |
| COPD             | 0        |
| CKD              | 4 (3.8)  |
| Neoplasia        | 2 (1.9)  |
| At ED presentation |        |
| PaO₂/FiO₂        | 281.4 (187.2–366.7) |
| LDH              | 374 (258.5–516.2) |
| CRP              | 89.2 (24.7–151.9) |
| Lymphocytes      | 1 (0.8–1.3) |
| Neutrophils      | 5.3 (3.7–8) |
| NLR              | 5.3 (3.5–8) |
| Creatinine       | 0.9 (0.7–1.1) |
| Transfer to ICU  | 34 (32.7) |
| Death            | 22 (21.2) |
| Neural biomarkers|          |
| NfL (pg/mL)      | 18.1 (12.6–35.1) |
| Tau (pg/mL)      | 0.5 (0.1–1.9) |
| GFAP (pg/mL)     | 197.7 (113.7–340.8) |
| UCH-L1 (pg/mL)   | 21.7 (14–33) |

Continuous variables were expressed as median (interquartile range), while categorical variables were expressed as count (percentage)

COVID-19 Coronavirus disease 2019, HTN arterial hypertension, CAD coronary artery disease, DM diabetes mellitus, COPD chronic obstructive pulmonary disease, CKD chronic kidney disease, ED Emergency Department, PaO₂/FiO₂ ratio of arterial oxygen partial pressure to fractional inspired oxygen, LDH lactic dehydrogenase, CRP C-reactive protein, NLR neutrophil to lymphocyte ratio, ICU intensive care unit, NfL neurofilament light chain, GFAP Glial fibrillary acidic protein, UCH-L1 ubiquitin carboxy-terminal hydrolase L1

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are especially increased in patients with neurological symp-
toms [19, 20]. Other groups examined the cerebrospinal fluid
demonstrating the absence of cytokine storm or neuroin-
flammatory markers in most examined samples [21]. In a
cohort of healthcare workers, blood NfL levels have been
reported to be increased in mild to moderate COVID-19
patients as compared to SARS-CoV-2 negative colleagues,
indicating that NfL increase in the blood is associated to
SARS-CoV-2 infection [22]. The study was, however,
not designed to assess the prognostic value of blood NfL
levels. Similarly, another study reported blood NfL to be
increased in SARS-CoV-2-infected as compared to non-
infected critically ill patients, suggesting a more pronounced
neuronal damage in COVID-19 patients [23]. This study
also indicated that higher NfL levels were associated with
unfavorable short-term outcome [23]. In a relatively small
study including as controls ICU patients with pneumonia of
other etiology, patients with COVID-19 were found to have
significantly higher blood GFAP levels. Furthermore, GFAP,
UCH-L1, and NfL positively correlated with Intensive Care
Delirium Screening Checklist score and were increased
in patients with delirium in the COVID-19 group but not
in the ICU controls [24]. In another study, NfL and GFP
blood levels measured at hospitalization were found to be
more elevated in patients with fatal outcome [25]. A small
study investigating a large number of biomarkers, has found
elevated total tau levels in blood extracellular vesicles in
COVID-19 patients as compared to historical controls [26].
Finally, no associations were found between NfL, GFAP,
UCH-L1 or tau and respiratory function or cytokines in

Fig. 1 Biomarkers of neural damage are increased at admission in
patients that will decrease from COVID-19. NfL (A), GFAP (B),
and tau (D), but not UCH-L1 (C), plasmatic levels are significantly
increased at admission in the ER in patients with fatal outcome
(dead) of COVID-19 as compared to survivor (alive). Only NfL (E)
and UCH-L1 (G), are significantly increased in patients that were
transferred to ICU (ICU) as compared to those that were not (non
ICU), while GFAP (F), and tau (H) are unchanged. Boxes and whisk-
ers represent median and 95% confidence interval values. * ≤ 0.05,
** ≤ 0.01, *** ≤ 0.001 (Mann–Whitney)
COVID-19 patients [24]. The measures in all these studies were performed in samples not obtained early at hospital admission, but rather during disease course or even in the ICU. This inevitably prevented from clearly establishing the predictive value of these neural markers in terms of clinical outcome.

In this scenario, we sought to understand whether CNS tissue damage biomarkers are early predictors of...
COVID-19 disease evolution. We found that blood NfL, GFAP, UCH-L1, and tau levels are elevated in patients with fatal outcome (UCH-L1 approaching but not reaching statistical significance), while data were weaker considering ICU admission. This latter inconsistency may derive from ICU admission being a less informative and objective endpoint. Despite this is a monocentric study ensuring homogeneity in terms of clinical protocols and decision making, criteria have slightly changed along the pandemic, a variety of different circumstances lead to ICU transfer. We found that NfL and tau are the best predictors of fatality according to ROC curves, and considering them together or in different combinations does not improve predictive accuracy. Patients with higher tau blood levels showed significantly higher mortality rates compared with patients with lower levels of the protein. Further analyses are needed to verify whether tau or NfL increase the predictive power of other, more conventional, parameters, with their ability to estimate nervous system damage, a tissue that is especially sensitive to hypoxia. The good correlations we found with conventional biomarkers is promising. These results are not readily transferable at the individual level to predict disease course, but may add value when used in combination with other, now more established, biomarkers.

NfL and UCH-L1 are expressed in both central and peripheral nervous system, while GFAP and tau are more exclusive of the central nervous system. The fairly good concordance of NfL, GFAP, and tau data may indicate a preferential central origin of these proteins in COVID-19 patients. We do not have data on a control cohort affected by acute respiratory distress of different origin, or a different severe infectious disease, thus we cannot attribute these findings specifically to SARS-CoV-2 infection.

In conclusion, the finding that NfL and tau blood levels measured at admission to the ED predict COVID-19 course and possibly fatality points to the potential clinical usefulness of these biomarkers in the prognostic work-up along with conventional biomarkers, to anticipate and ensure proper handling of more severe forms of the disease.

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