Observational cohort study of neurological involvement among patients with SARS-CoV-2 infection

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Ther Adv Neurol Disord

2021, Vol. 14: 1–14

DOI: 10.1177/1756286421993701

Abstract

Background: A growing number of reports suggest that infection with SARS-CoV-2 often leads to neurological involvement; however, data on the incidence and severity are limited to mainly case reports and retrospective studies.

Methods: This prospective, cross-sectional study of 102 SARS-CoV-2 PCR positive patients investigated the frequency, type, severity and risk factors as well as underlying pathophysiological mechanisms of neurological involvement (NIV) in COVID-19 patients.

Results: Across the cohort, 59.8% of patients had NIV. Unspecific NIV was suffered by 24.5%, mainly general weakness and cognitive decline or delirium. Mild NIV was found in 9.8%; most commonly, impaired taste or smell. Severe NIV was present in 23.5%; half of these suffered cerebral ischaemia. Incidence of NIV increased with respiratory symptoms of COVID-19. Mortality was higher with increasing NIV severity. Notably, 83.3% with severe NIV had a pre-existing neurological co-morbidity. All cerebrospinal fluid (CSF) samples were negative for SARS-CoV-2 RNA, and SARS-CoV-2 antibody quotient did not suggest intrathecal antibody synthesis. Of the patients with severe NIV, 50% had blood–brain barrier (BBB) disruption and showed a trend of elevated interleukin levels in CSF. Antibodies against neuronal and glial epitopes were detected in 35% of the patients tested.

Conclusion: Cerebrovascular events were the most frequent severe NIV and severe NIV was associated with high mortality. Incidence of NIV increased with respiratory symptoms and NIV and pre-existing neurological morbidities were independent risk factors for fatality. Inflammatory involvement due to BBB disruption and cytokine release drives NIV, rather than direct viral invasion. These findings might help physicians define a further patient group requiring particular attention during the pandemic.

Keywords: Neuro-COVID, Cerebrovascular events, COVID-19

Received: 8 November 2020; revised manuscript accepted: 13 January 2021.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affects the lower respiratory tract causing the core clinical symptoms of the coronavirus disease 2019 (COVID-19). However, there is growing evidence that infection with SARS-CoV-2 often leads to neurological signs and symptoms. Cohort studies have reported that up to 30% of hospitalized COVID-19 patients displayed at least one neurological symptom,[1] including cerebrovascular events, meningoencephalitis or viral associated encephalopathy.[2–4] Beyond these severe neurological manifestations, further neurological signs and symptoms including headache, dizziness, confusion, myalgia, and fatigue[5–7] have been described. Neurological involvement (NIV) in COVID-19 may be common and was recently termed NeuroCOVID. However, the precise frequency and severity of the neurological effects of COVID-19 remain elusive and further

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Table 1. Characteristics of the study cohort.

Age, years	
Median ± SD	61.5 ± 16.7
IQR	53.5 – 73.0
Gender – *n* (%)	
Male	71 (70.0)
Female	31 (30.0)
Medical history, pre-existing conditions – *n* (%)	
Neurological diseases	41 (40.2)
Cardiovascular diseases, except arterial hypertension	37 (36.3)
Oncologic disease	20 (19.6)
Arterial hypertension	45 (44.1)
Diabetes	22 (21.6)
Pulmonary disease	29 (28.4)
Allergies	11 (10.8)
COVID-19 severity – *n* (%)	
No symptoms	2 (1.9)
Mild	24 (23.5)
Moderate	38 (37.2)
Severe	38 (37.2)

IQR, interquartile range.

information on the associated health burden is required. Current knowledge is mainly based on case reports or retrospective studies with small patient numbers, which did not dissect unspecific neurological symptoms and genuine neurological manifestation of COVID-19.

Furthermore, the pathophysiological disease mechanism is still under debate. SARS-CoV-2 might enter the brain through the olfactory nerves, especially given the anosmia in COVID-19, or through disruption of the blood–brain barrier (BBB) to exert direct effects.[8] Alternatively, immunologic effects may occur through autoantibodies, exuberant immune response or stress-mediated changes in gene expression,[5,9–11] as such autoimmune mechanisms have been described for other coronavirus-mediated complications.[12–14]

Here, we present the first prospective study of NIV among a cohort of 102 SARS-CoV-2 infected patients.

Material and methods

Study design, patients and procedures

This prospective trial was conducted to characterize NIV in a cohort of 102 SARS-CoV-2 positive patients (age range 20–95 years, median age 61.5 years; Table 1). Inpatients with SARS-CoV-2 infection were included.

All patients were recruited at the University Hospital of Essen over a period of 4 months (April–July 2020). All patients underwent a structured clinical and neurological examination and were classified for COVID-19 disease severity and NIV as described below. Pre-existing medical conditions, ongoing medication, medical history and course of the disease were obtained from direct questioning/observation or medical files. Blood samples were obtained from all patients; lumbar puncture and electroencephalogram were performed for medical purposes.

Detection of SARS-CoV-2, classification of COVID-19 severity and neurological involvement

Only SARS-CoV-2 positive patients were included in the study cohort. SARS-CoV-2 was detected in nasopharyngeal swaps or in bronchoalveolar lavage fluid by PCR analysis (MagNA Pure 96 DNA and Viral NA Small or Large Volume Kit using MagNA Pure 96, both Roche, Rotkreuz, Switzerland and RealStar SARS-CoV-2 RT-PCR Kit 1.0, Altona Diagnostics GmbH, Hamburg, Germany using CFX 96, Biorad, München, Germany) following the manufacturers' instructions. Antibody testing was performed in blood and cerebrospinal fluid (CSF) samples using anti-SARS-CoV-2 IgG ELISA (EUROIMMUN AG, Lübeck, Germany). The result is expressed semi-quantitatively as ratio (extinction probe/extinction calibrator).

COVID-19 severity was categorized in terms of pulmonary symptoms into asymptomatic, mild, moderate and severe as described in Buonsenso *et al.*[15] (Table 2).

All patients underwent structured neurological examination by trained neurologists. Anosmia

Table 2. Definition of COVID-19 severity (modified according to Buonsenso *et al.*[15]).

Mild COVID-19
Positive RT-PCR test for SARS-CoV-2
Upper respiratory symptoms (e.g. pharyngeal congestion, sore throat and fever) for a short duration or asymptomatic infection
No abnormal radiographic and septic presentation
Moderate COVID-19
Positive RT-PCR test for SARS-CoV-2
Mild pneumonia
Symptoms such as fever, cough, fatigue, headache and myalgia
Severe COVID-19
Positive RT-PCR test for SARS-CoV-2
Mild or moderate clinical features, plus any manifestations that suggest disease progression
Respiratory failure with need for mechanical ventilation (e.g. acute respiratory distress syndrome, persistent hypoxia that cannot be alleviated by inhalation through nasal catheters or masks) Septic shock
Organ failure that needs to be monitored in the intensive care unit

Table 3. Categorization of neurological involvement (NIV) in COVID-19 patients.

No NIV
No evidence of neurological symptoms assessed by clinical examination and anamnestic evaluation
Unspecific NIV
For example, dizziness, headache, worsening of a pre-existing neurological condition, muscle pain, general weakness, all with onset between 10 days prior to and 20 days after a positive SARS-CoV-2 PCR or after onset of COVID symptoms
Mild NIV
For example, impaired taste or smell, cranial nerve paresis. All with onset between 10 days prior to and 20 days after a positive SARS-CoV-2 PCR or after onset of COVID symptoms
Severe NIV
For example, acute cerebrovascular disease, seizure, myopathy or neuropathy. All with onset between 10 days prior to and 20 days after a positive SARS-CoV-2 PCR or after onset of COVID symptoms

was detected using Quick Smell Identification Test™ (Sensonics, Inc., Haddon Heights, NJ, USA). Neurological symptoms were regarded as SARS-CoV-2 associated if they had an onset between 10 days prior to and 20 days after a positive SARS-CoV-2 PCR test or after onset of COVID-19 symptoms. Neurological signs and symptoms that had occurred earlier than 10 days prior to onset were classified as 'pre-existing'. Neurological signs and symptoms were defined and categorized into four groups: no NIV, unspecific, mild and severe NIV (Table 3).

Blood samples and spinal fluid specimen

Peripheral blood and CSF were analysed for routine parameters [leukocytes cell count (number/µl), level of creatinine kinases (U/l),

D-dimer (mg/l), lactate dehydrogenase (U/l), lactate (mmol/l), glucose (mmol/l) and C-reactive protein (mg/l), levels of heavy chain neurofilament (ng/ml) as well as the occurrence of a broad spectrum of neuronal antibodies and antibodies against myoproteins]. Blood and CSF parameters were assessed in the central laboratory facility of the University Hospital Essen. Levels of heavy chain neurofilament (ng/ml) were determined in serum and spinal fluid at the laboratory facility EUROIMMUN AG, Lübeck, Germany. An ELISA test (EUROIMMUN AG) was used to measure neurofilament concentration with a detection limit in serum of 0.027 ng/ml and the functional sensitivity of 0.117 ng/ml. Neuronal antibodies against Hu, Ri, ANNA-3, Yo, Tr/DNER, myelin, Ma/Ta, GAD65, amphiphysin, aquaporin-4, glutamate-receptors (Typ NMDA, AMPA), GABA/B receptor, LGI1, CASPR2, ZIC4, DPPX, glycine receptor, mGluR1, mGluR5, Rho-GTPase activating protein 25, ITPR1, Homer 3, MOG, recoverin, neurochondrin, GluRD2, flotillin 1/2, IgLON5, CV2, PNMA2, SOX1, titin and Zic4 were determined in serum of patients. Concentrations of antibodies against myelin basic protein, gangliosides (GM1, GM2, GM3, GD1a, GD1b, GT1b, GO1b) were measured. Furthermore, we screened for antibodies against MDA5, NXP2, SAE1, Mi-2alpha, Mi-2beta, TIF1gamma, Ku, PM75, SRP, Pl-7, PL-12, EJ, OJ, Ro-52 and cN-1A. Antibody diagnostics were performed at the laboratory facility EUROIMMUN AG, Lübeck, Germany using test strips with coated membrane chips of purified, biochemically characterized antigens. Cytokine concentrations (IL-6, IL-8 and TNF-alpha) were determined using magnetic bead sandwich immunoassay (Bio-Plex Pro, Human Cytokin Screening Assay, Biorad, Germany) according to the manufacturer's protocol. Optical density was measured with Bio-Plex 200 (Biorad, Germany).

Statistics

All data are presented as mean, wherever possible with standard error of the mean, and p values, which were calculated using GraphPad Prism software version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). Group differences were assessed using one-way analysis of variance (Kruskal–Wallis) with Bonferroni's multiple comparison *post-hoc* tests, after analysing for parametric distribution with Kolmogorov–Smirnov test. Pearson's chi-square test was used for categorical comparisons. A p value < 0.05 was considered to be significant.

Standard protocol approvals, registrations and patient consents

The study was performed in accordance with the principles of the Declaration of Helsinki and the local Ethics Committees approved the study plan (Ethics Committee University of Duisburg-Essen, approval number: 20-9284-BO). Signed informed consent was obtained. The study is registered in the German register for clinical trials (DRKS) under the registration number: DRKS00023312.

Results

Among 102 patients with COVID-19 included in the study, 61 (59.8%) were found to have any neurological signs or symptoms. The distribution of neurological signs and symptoms among this cohort is shown below (Table 4).

It was found that 25 (24.5%) patients suffered unspecific NIV. Within this group, eight patients (32%) exhibited a reduction of mobility or general weakness, six (24%) had a psychiatric disorder including delirium. Worsening of a pre-existing neurological condition or cognitive decline was present in five (20%) patients and severe headache was found in one (4%) case.

In the whole cohort, 10 patients (9.8%) suffered mild NIV. Within this group, impaired taste or smell was most common ($n = 9$, 90%), followed by transient ischaemic attack (TIA, $n = 1$, 1%).

In the whole study population, severe NIV was present in 24 patients (23.5%). Of these patients with severe NIV, half suffered cerebral ischaemia ($n = 12$), five (20.8%) experienced cerebral bleeding, three (12%) had seizures, and encephalitis and myopathy/neuropathy each occurred in two (8.3%) cases.

COVID-19 patients were categorized by the severity of their respiratory symptoms. Increasing severity of respiratory symptoms was associated with increasing NIV severity (Figure 1A).

Of the patients with mild COVID-19, 10 (42%) had NIV (unspecific, mild or severe). Of the patients with a moderate COVID-19 course of

Table 4. Neurological signs and symptoms in the cohort.

Neurological involvement			
		***n* (% of NIV)**	***n* (% of total)**
Unspecific	Worsening of pre-existing neurological deficit	5 (8.5)	
	Reduction of mobility/general weakness	8 (13.6)	
	Psychiatric disorder including delirium	6 (10.2)	
	Severe headache	1 (1.7)	
	Cognitive decline	5 (8.5)	
	Total		25 (24.5)
Mild	Impaired taste or smell	9 (15.3)	
	Transient ischaemic attack	1 (1.7)	
	Total		10 (9.8)
Severe	Cerebral bleeding	5 (8.5)	
	Cerebral ischaemia	12 (20.3)	
	Seizure	3 (5.1)	
	Encephalitis	2 (3.4)	
	Neuropathy/myopathy	2 (3.4)	
	Total		24 (23.5)

NIV, neurological involvement.

disease (mainly moderate pneumonia), 19 (50.1%) showed NIV, and 30 (79%) of patients with severe COVID-19 (referring to patients with respiratory failure and the need for mechanical ventilation) had any NIV. Numerical increase in NIV with increasing respiratory COVID-19 severity was detected due to cases of severe and unspecific NIV. Thus, among patients with mild COVID-19, four (14.7%) had unspecific NIV and one (4.2%) had severe NIV. Of patients with moderate COVID-19, 19 (39%) had unspecific NIV and seven (18.4%) had severe NIV. In patients with severe COVID-19, 13 (34.2%) had unspecific NIV and 16 (42%) presented with severe NIV. Thus, the incidence of NIV increases with the severity of respiratory COVID-19.

Mortality increased with increasing NIV severity (Figure 1B). For patients without neurological symptoms, mortality was calculated to be 14.3%; among patients with unspecific NIV, mortality was 32% and in patients with severe NIV mortality was found to be 41.7%. None of the patients with mild NIV died in the current study (Figure 1B). Among all patients with a fatal outcome, 41.7% had severe NIV (Figure 1B, inlay). Severe NIV is thus an independent risk factor for fatality.

Average age did not differ significantly between patients with no, unspecific, mild or severe NIV (no neurological symptoms $n=41$, average age 60 years; unspecific NIV, $n=25$, average age 61 years; mild NIV, $n=10$, average age 51 years; and severe NIV, $n=24$, average age 62 years; Figure 1C).

We further investigated the relationship between pre-existing neurological co-morbidities and the presence or severity of NIV and found increasing levels of neurological severity among those patients with pre-existing neurological co-morbidities (Figure 1D). In patients without NIV, we found

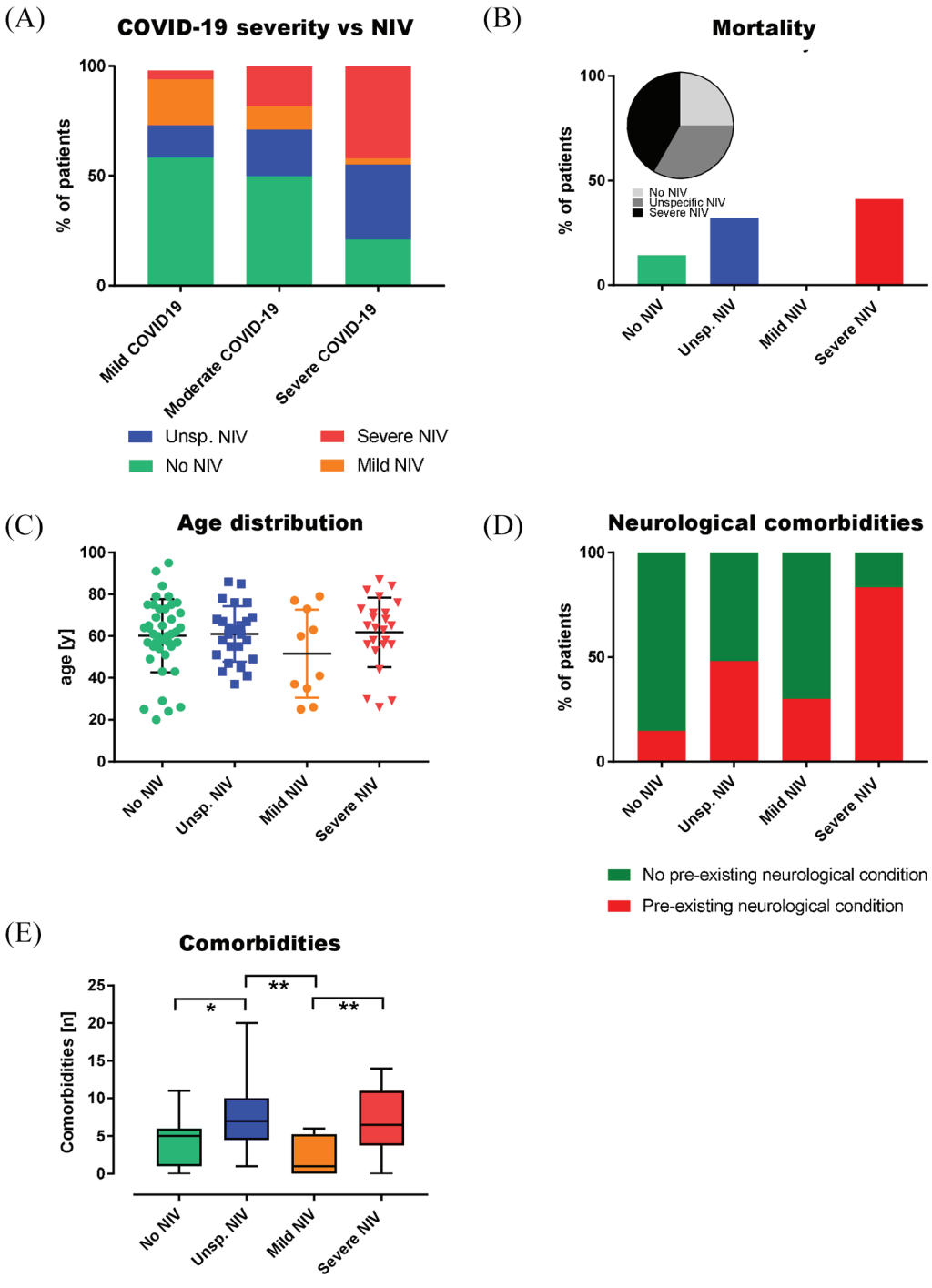

Figure 1. Clinical characteristics of the COVID-19 cohort.
(A) Proportion of patients showing unspecific, mild focal and severe focal neurological symptoms according to severity of COVID-19 classified as mild, moderate or severe. Proportion of patients (%) showing each type of neurological symptom grouped within the categories of unspecific, mild focal and severe focal neurological symptoms. (B) Mortality (% of patients) in relation to severity of COVID-19 and neurological involvement (none, unspecific, mild focal or severe focal). Inlays show all deaths, and proportion of NIV classification and COVID-19 severity. (C) Distribution of age in relation to neurological involvement categories (none, unspecific, mild focal or severe focal). (D) Proportion of patients (%) who had pre-existing neurological morbidity within each of the neurological involvement categories (none, unspecific, mild focal or severe focal). (E) Number of comorbidities in relation to neurological involvement categories. Classification *$p < 0.05$, **$p < 0.01$. (none, unspecific, mild focal or severe focal).
NIV, neurological involvement; Unsp., unspecific.

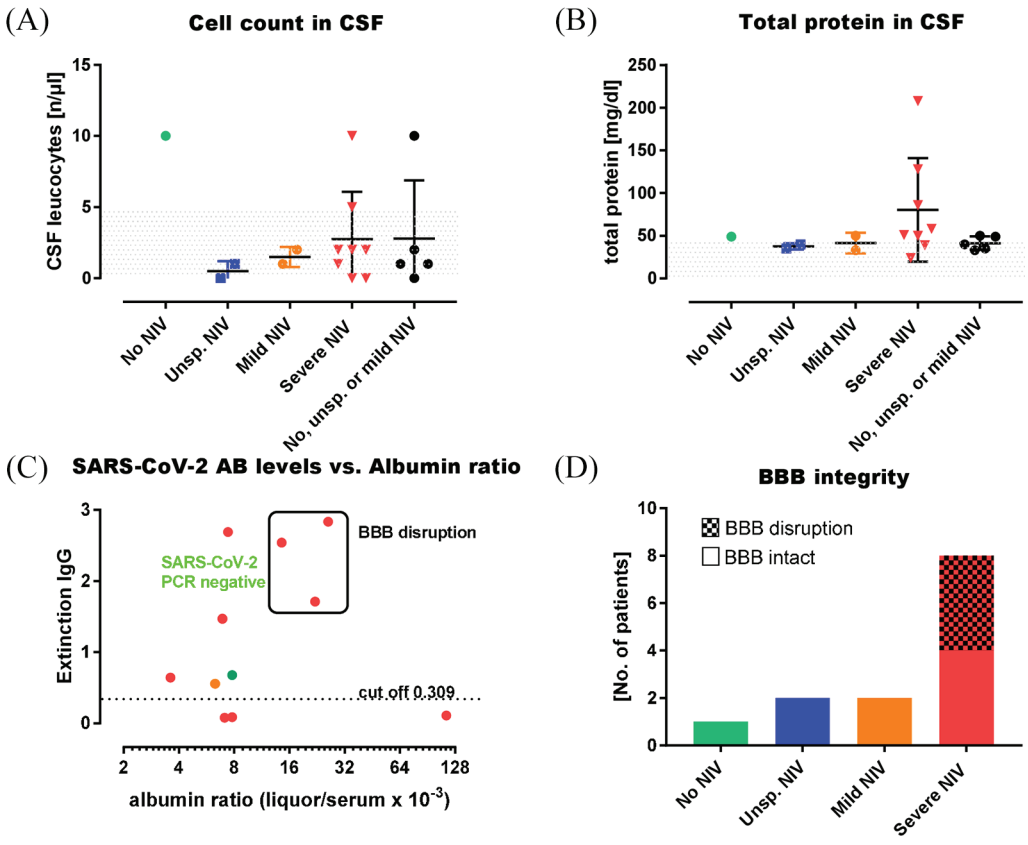

Figure 2. Cerebrospinal fluid (CSF) analysis of COVID-19 patients.
(A) and (B) CSF cell counts (*n*/µl) and level of CSF protein (mg/dl) according to neurological involvement categories (none, unspecific, mild focal or severe focal). (C) Extinction of IgG antibody against SARS-CoV-2 against albumin ratio. The colour-coding shows each of the neurological symptom categories: none (green), mild focal (orange) or severe focal (red). (D) Integrity of the blood–brain barrier (BBB), graphed by number of patients who showed a disruption or an intact BBB within each of the neurological involvement categories (none, unspecific, mild focal or severe focal).
AB, antibody; NIV, neurological involvement; Unsp., unspecific.

that only six (14.6%) had a pre-existing neurological co-morbidity; in patients with unspecific NIV, 12 (48%) had a pre-existing co-morbidity; three (30%) patients with mild NIV and 20 patients (83.3%) with severe NIV had a pre-existing neurological co-morbidity.

Additionally, the number of pre-existing co-morbidities in general (not only neurological co-morbidities) was higher in patients with unspecific NIV, compared with patients without any NIV ($p=0.01$) and compared with patients with mild NIV ($p=0.002$). Furthermore, patients with severe NIV had significantly more pre-existing co-morbidities compared with patients with mild NIV ($p=0.009$; Figure 1E). Co-morbidities thus predispose for NIV.

We further assessed potentially underlying mechanisms of NIV in COVID-19 patients. For this, CSF was analysed in 13 patients and blood analyses were performed.

CSF cell leucocyte counts did not show significant differences between groups stratified for NIV (total $n=13$ samples; Figure 2A). Although total protein (total number of samples $n=13$) was increased in 46% of patients with severe NIV, alterations did not reach the level of statistical significance (Figure 2B). We found that all patients showed oligoclonal bands Type 4 (identical oligoclonal bands in CSF and serum) except one patient with type 1 pattern (no bands in CSF and serum). Elevated intrathecal immunoglobulin fractions were not found in any patient (data not shown).

To test for a direct viral central nervous system invasion, we tested CSF samples for SARS-CoV-2 RNA using RT-PCR. All samples tested were

found negative for SARS-CoV-2 RNA ($n=13$). We furthermore performed antibody testing for SARS-CoV-2 S1 IgG in patients' CSF. Samples from all patients tested revealed low levels of SARS-CoV-2 IgG in CSF, compared with serum IgG (on average 4.68-fold higher in serum); three samples were under the extinction cut-off. Three of the patients with high SARS-CoV-2 IgG extinction concomitantly had high albumin serum/CSF ratio and an age defined [age(y)/15+4] BBB disruption ($n=10$; Figure 2C).

Due to this finding, we correlated the presence and severity of NIV to a disruption of the BBB. BBB integrity was neither compromised in the patient without neurological symptoms ($n=1$) nor in patients with unspecific NIV ($n=2$) or mild NIV ($n=2$), but 50% of patients with severe NIV ($n=8$) presented with a disruption of the BBB (Figure 2D). Inflammatory involvement of the CSF due to BBB disruption may thus partially drive NIV.

To investigate the underlying mechanism, we analysed inflammatory cytokines in the CSF. A trend towards elevated levels of IL-6, IL-8 and TNF-alpha was seen in patients with severe NIV compared with no, unspecific or mild NIV, but did not reaching the level of significance ($n=12$; Figure 3A–C). BBB integrity was assessed according to albumin quotient in CSF and IL-8 increase was significantly correlated with BBB disruption ($p=<0.05$). Patients with BBB disruption were found to show a trend towards higher levels of IL-6 and TNF-alpha but this did not reach the level of significance (Figure 3A–C; inlays).

To evaluate autoimmune mediated mechanisms that may facilitate NIV in COVID-19, various antibodies against neuronal or glial proteins (see Methods) were analysed in serum samples (Figure 3D). Overall, 35% of COVID-19 patients who were tested for the presence of neuronal or glial antibodies showed positivity for at least one antibody. We found antibodies against titin, MOG, Yo, Mi2a/2b, neuronal-antigen and DNER, without any association with clinical presentation (Table 5). There was no significant difference between NIV groups. Positivity was found in 45.5% of patients with no NIV ($n=11$); 50% of patients with unspecific NIV ($n=4$); 20% of patients with mild NIV ($n=5$); and 35% of patients with severe NIV ($n=11$).

Analysis of CSF and blood neurofilament (heavy chain) concentrations did not show significant differences between NIV groups (Figure 3E and F).

Discussion

In this study we prospectively enrolled 102 patients who were positive for SARS-CoV-2 to investigate the nature, frequency and severity of neurological complications of COVID-19. We identified pre-disposing factors for NIV and suggest inflammatory-mediated mechanisms driving neurological manifestations of COVID-19.

We found stroke to be the most frequent severe neurological complication of COVID-19. In this study, 12% of all patients suffered ischaemic stroke including half of all severe NIV cases. This number is higher than that found in previously published retrospective studies, which reported that 5% of COVID-19 patients suffered ischaemic stroke,[1] and higher than a meta-analysis of 10 studies, which found an incidence of 1.8%.[16] Intracerebral haemorrhage was found to be the second most commonly occurring severe NIV in COVID-19 patients; three out of five events occurred during systemic anticoagulation required for extracorporeal membrane oxygenation (ECMO) therapy.[17] Despite the fact that haemorrhages have been reported in other viral conditions such as in H3N2 influenza pneumonia[18] and direct association with SARS-CoV-2 has been discussed before,[19] our data suggest ECMO therapy as the predominant cause. The clinical relevance of this study is underlined not just by the high frequency of severe NIV, but also by the finding that severe NIV tripled mortality compared with patients without NIV.

In contrast to previous findings,[20] we found severity of respiratory symptoms in COVID-19 as a key pre-disposing factor for the development of severe NIV. As pre-existing neurological conditions accumulate with increasing age, the average age could be excluded as a risk factor for developing severe NIV.[1,21]

Beside these severe NIV endpoints, we furthermore monitored mild neurological signs and symptoms, mainly ageusia and anosmia, which was found in 8.8% of all cases, the frequency of which ranges from 6% to 88% in the literature.[1,22–24] As these signs and symptoms were discussed as the most common forms of NIV in COVID-19,[25] a pre-selection bias of our mono-centre university

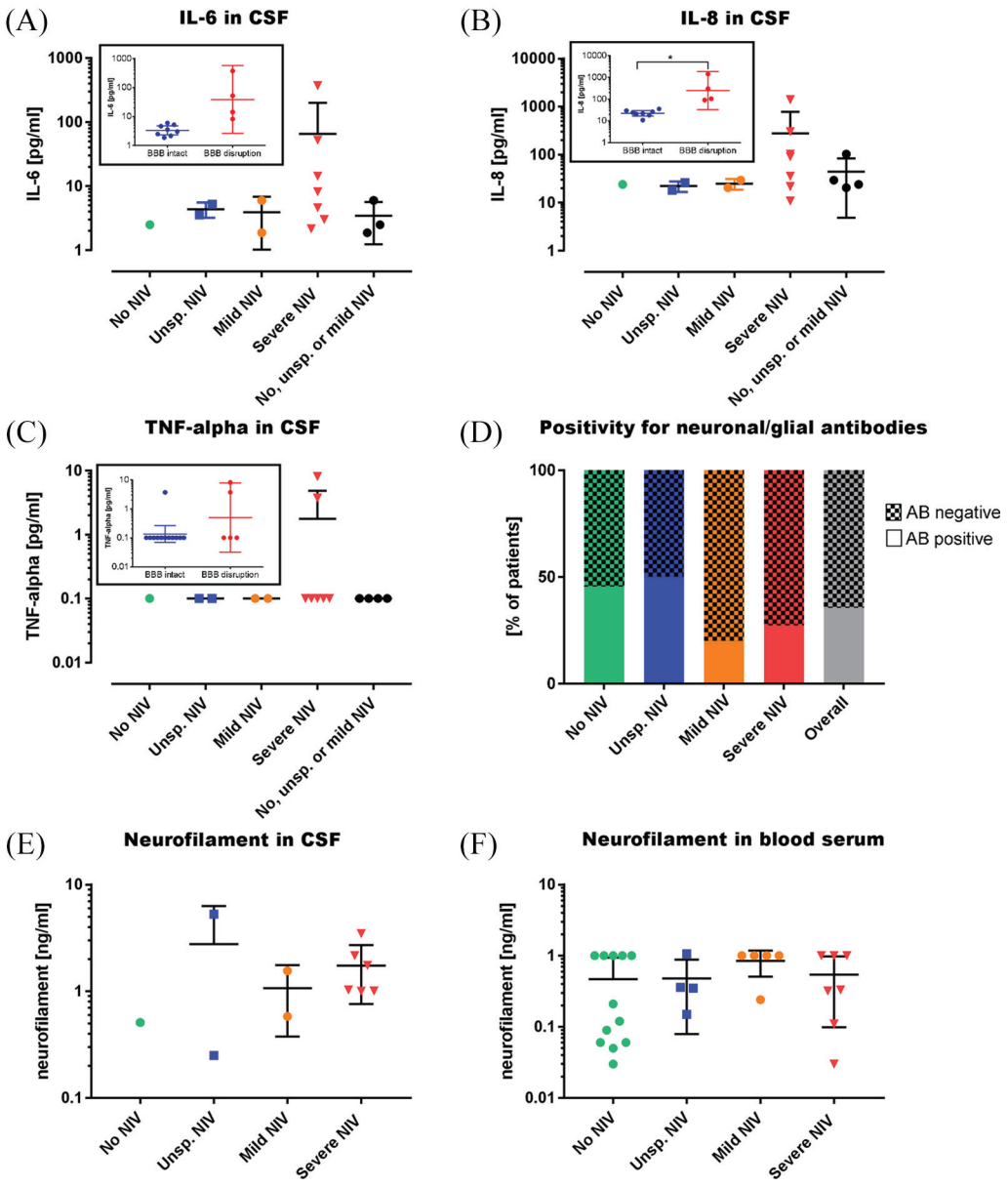

Figure 3. Cerebrospinal fluid (CSF) and blood analysis of COVID-19 patients.
(A)–(C). Concentration of IL-6, IL-8, TNF-alpha (pg/ml) in the CSF by neurological symptom involvement (none, unspecific, mild focal or severe focal classification $*p < 0.05$). Inlays: respective status of blood–brain barrier (BBB) in relation to interleukin level. (D) Antibodies against neuronal or glial epitopes in serum in relation to neurological involvement categories (none, unspecific, mild focal or severe focal). (E) Measurement of neurofilament in CSF grouped according to each of the neurological involvement categories (none, unspecific, mild focal or severe focal). (F) Measurement of neurofilament in serum grouped according to each of the neurological involvement categories (none, unspecific, mild focal or severe focal).
AB, antibody; NIV, neurological involvement; Unsp., unspecific; IL, interleukin.

hospital study may have been overestimation of severe cases and underestimation of mild NIV.

We separately assessed unspecific NIV – as a critical advance in scientific knowledge compared with previous studies – and found unspecific NIV, such as cognitive decline, muscular weakness, delirium and impaired consciousness, in 24.5% of all COVID-19 cases in this cohort. This finding has significant clinical relevance, showing that increased mortality is not only contributed to by severe NIV but also by the presence of unspecific NIV. Unspecific NIV is associated with a doubling in mortality compared with patients without

Table 5. Antibodies against neuronal epitopes and corresponding COVID-19 severity and neurological involvement.

Auto antibody epitopes	COVID-19 severity	Neurological involvement severity	Neurological symptom
Titin	Severe	Severe	Seizures, myopathy
Neuronal antigen	Mild	Unspecific	Mild cognitive deficit
Neuronal antigen	Moderate	Unspecific	Severe headache
PL7, MOG IgG	Mild	Mild	Dysphagia
Yo	Severe	Severe	Cerebral ischaemia
Mi-2a	Moderate	Unspecific	Severe headache
MOG IgG	Severe	Severe	Cerebral bleeding
Mi-2b	Moderate	Unspecific	Severe headache
Neuronal antigen	Mild	Mild	Impaired smell/taste
DNER	Mild	Mild	Impaired smell/taste

NIV. In relation to the role of pre-existing neurological conditions, we found them increased in patients with unspecific NIV and as major risk factor for the development of severe NIV.

The proportion of patients with delirium and cognitive decline in this cohort was high, at almost 19%, comparable to that reported in former studies, which describe confusion and agitation in up to 69% of patients[7] and impaired consciousness in 14% of patients.[1] Remarkably, this high proportion of patients with unspecific NIV resembles other viral respiratory conditions, such as H1N1, where mild neurological signs and symptoms, most often headache, numbness, paraesthesia, drowsiness and coma,[26–28] are reported in up to 42% of patients. As previously suggested, we considered the assertion that impaired consciousness was related to epileptic activity.[6,24] Indeed, we found seizures in 2.9% of all COVID-19 patients, but no relevant alterations in electroencephalography were recorded (Supplemental Material results online) in patients with impaired consciousness or cognitive decline in our study.

To further delineate the pathophysiologic mechanism underlying NIV in COVID-19 patients we analysed CSF and blood samples. Viral RNA was not detectable in CSF of patients presenting with neurological signs and symptoms. Despite a lot of effort to detect SARS-CoV-2 RNA in CSF, most studies could not detect any or found evidence in a few individuals.[7,29–32] A review article suggests a positive detection of SARS-CoV-2 in 1.28% of the cases among 1018 pooled cases. This result was calculated by including 13 studies reporting positive results in 13 of 67 patients, and 43 reported negative results in 951 patients.[33]

In line with our negative PCR results, antibody indices for SARS-CoV-2 did not suggest intrathecal antibody production and argue against cerebral virus replication and cerebral viral invasion.

Still, we cannot completely rule out direct central nervous system infection with SARS-CoV-2 in our study. One limitation is the low patient numbers included in the CSF analysis. Also, the fact that increased leucocytes were found in three patients and SARS-CoV-2 specific antibodies were found in some, despite the fact that these patients mainly were found with BBB disruption, indicates that further studies are needed to address this question.

We found pleocytosis in the CSF of some patients with severe NIV, elevated inflammatory cytokines and BBB disruption in half of all patients with severe NIV. Angiotensin converting enzyme 2, as it binds to SARS-CoV-2,[34] is ubiquitously expressed on epithelia and brain tissue.[35,36] Receptor binding can cause a storm of cytokines,[11,37] which may also explain BBB disruption in our

patients.[38] Back to bench side, an increase of pro-inflammatory cytokines and BBB disruption triggers coagulation,[37] and may further explain the high incidence of stroke in COVID-19 patients. The interaction between inflammation and vessel clots has been described for other conditions.[39,40] BBB disruption – particularly in the temporal lobe – can further facilitate seizures[41] and cognitive decline.[42] Cytokines are also known to affect the peripheral nervous system and consecutive demyelination[43] and lead to further organ damage.[44]

We assessed the question whether autoimmune phenomena may further influence the severity and incidence of NIV in SARS-CoV-2 positive patients, as previously considered in relation to Middle East Respiratory Syndrome.[45] Indeed, a remarkably high proportion of patients (35%) showed positivity for at least one of the antibodies tested against neuronal epitopes.

As there was no correlation between clinical presentation and the detection of neuronal antibodies and no correlation between antibody frequency and NIV frequency/severity, we assume that the detection of these antibodies represents a general immune activation in COVID-19 patients. The inflammatory stimulus of SARS-CoV-2 and the high cytokine release may lead to unmasking of epitopes by unspecific epitope spreading. CSF and blood analysis within our study suggest that BBB disruption and cytokine release as well as autoimmune mechanisms are involved in development of NIV in COVID-19.

As a single centre analysis, this study is limited by the low patient numbers in some of the subgroups, selection bias and the lack of longitudinal long-term data. Despite these limitations, compared with other previous studies that investigated NIV, we present the first prospective cohort of only SARS-CoV-2 PCR positive patients.

We identified clinical risk factors for severe NIV and CSF changes that provide evidence for indirect inflammatory mechanisms driving neurological manifestations of COVID-19 infection.

Author contributions

Michael Fleischer: major role in acquisition and analysing data; Martin Köhrmann: revised the manuscript for intellectual content; Sebastian Dolff: interpreted the data; revised the manuscript for intellectual content; Fabian Szepanowski: revised the manuscript for intellectual content; Karsten Schmidt: major role in acquisition of the data; Frank Herbstreit: revised the manuscript for intellectual content; Cansu Güngör major role in acquisition of the data; Ben Stolte: major role in acquisition of the data; Christine Stadtler: major role in acquisition of the data; Joachim Rißе: major role in acquisition of the data; Oliver Witzke: interpreted the data; revised the manuscript for intellectual content; Melanie Fiedler: major role in acquisition of the data; Anne-Kathrin Mausberg: revised the manuscript for intellectual content; Clemens Kill: major role in acquisition of the data; Gerd Meyer zu Horste: interpreted the data; revised the manuscript for intellectual content; Katharina Steiner: major role in acquisition of the data; Michael Forsting: revised the manuscript for intellectual content; Ulrich Sure: revised the manuscript for intellectual content; Ulf Dittmer: interpreted the data; revised the manuscript for intellectual content; Torsten Brenner: revised the manuscript for intellectual content; Christoph Kleinschnitz: design and conceptualized study; analysed the data; drafted the manuscript for intellectual content; Mark Stettner: design and conceptualized study; analysed the data; drafted the manuscript for intellectual content.

Conflict of interest

M. Fleischer reports no disclosures relevant to the manuscript.

M. Köhrmann reports no disclosures relevant to the manuscript.

S. Dolff reports no disclosures relevant to the manuscript.

F. Szepanowski reports no disclosures relevant to the manuscript.

K. Schmidt reports no disclosures relevant to the manuscript.

F. Herbstreit has received speaker honoraria from Biotest and Maquet Getinge.

C. Güngör reports no disclosures relevant to the manuscript.

B. Stolte reports no disclosures relevant to the manuscript.

Ch. Stadtler reports no disclosures relevant to the manuscript.

J. Rißе reports no disclosures relevant to the manuscript.

O. Witzke has received research grants for clinical studies, speaker's fees, honoraria and travel expenses from Amgen, Alexion, Astellas, Basilea, Biotest, Bristol-Myers Squibb, Correvio, Chiesi, Gilead, Hexal, Janssen, Dr. F. Köhler Chemie, MSD, Novartis, Roche, Pfizer, Sanofi, TEVA and UCB.

M. Fiedler reports no disclosures relevant to the manuscript.

A. Mausberg reports no disclosures relevant to the manuscript.

C. Kill reports no disclosures relevant to the manuscript.

G. Meyer zu Horste has received speaker honoraria and served in advisory boards for LFB Pharma.

K. Steiner reports no disclosures relevant to the manuscript.

M. Forsting reports no disclosures relevant to the manuscript.

U. Sure reports no disclosures relevant to the manuscript.

U. Dittmer reports no disclosures relevant to the manuscript.

T. Brenner reports no disclosures relevant to the manuscript.

C. Kleinschnitz reports no disclosures relevant to the manuscript.

M. Stettner served on the scientific advisory and/or received speaker honoraria or travel funding from UCB, Biogen Idec; Grifols, Genzyme, Roche, Merck, Novartis, Octapharma, Sanofi-Aventis, TEVA, and Bayer Healthcare.

All authors declare approval of the version that is to be published.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Funding was obtained from the Foundation of the University Hospital Essen (Stiftung Universitätsmedizin Essen).

Supplemental material

Supplemental material for this article is available online.

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References

1. Mao L, Jin H, Wang M, *et al.* Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol* 2020; 77: 683–690.
2. Werner C, Scullen T, Mathkour M, *et al.* Neurological impact of coronavirus disease of 2019: practical considerations for the neuroscience community. *World Neurosurg* 2020; 139: 344–354.
3. Poyiadji N, Shahin G, Noujaim D, *et al.* COVID-19-associated acute hemorrhagic necrotizing encephalopathy: CT and MRI features. *Radiology* 2020; 296: E119–E120.
4. Wong PF, Craik S, Newman P, *et al.* Lessons of the month 1: a case of rhombencephalitis as a rare complication of acute COVID-19 infection. *Clin Med (Lond)* 2020; 20: 293–294.
5. Li YC, Bai WZ and Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *J Med Virol* 2020; 92: 552–555.
6. Tsai ST, Lu MK, San S, *et al.* The neurologic manifestations of coronavirus disease 2019 pandemic: a systemic review. *Front Neurol* 2020; 11: 498.
7. Helms J, Kremer S, Merdji H, *et al.* Neurologic features in severe SARS-CoV-2 infection. *N Engl J Med* 2020; 382: 2268–2270.
8. Pouga L. Encephalitic syndrome and anosmia in COVID-19: do these clinical presentations really reflect SARS-CoV-2 neurotropism? A theory based on the review of 25 COVID-19 cases. *J Med Virol*. Epub ahead of print 16 July 2020. DOI: 10.1002/jmv.26309.
9. Desforges M, Le Coupanec A, Stodola JK, *et al.* Human coronaviruses: viral and cellular factors involved in neuroinvasiveness and neuropathogenesis. *Virus Res* 2014; 194: 145–158.
10. Blanco-Melo D, Nilsson-Payant BE, Liu WC, *et al.* Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell* 2020; 181: 1036–1045 e1039.
11. Mehta P, McAuley DF, Brown M, *et al.* COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; 395: 1033–1034.
12. Li H, Xue Q and Xu X. Involvement of the nervous system in SARS-CoV-2 infection. *Neurotox Res* 2020; 38: 1–7.
13. Arbour N, Day R, Newcombe J, *et al.* Neuroinvasion by human respiratory coronaviruses. *J Virol* 2000; 74: 8913–8921.

14. Desforges M, Le Coupanec A, Dubeau P, *et al.* Human coronaviruses and other respiratory viruses: underestimated opportunistic pathogens of the central nervous system? *Viruses* 2019; 12: 14.

15. Buonsenso D, Parri N, De Rose C, *et al.* Toward a clinically based classification of disease severity for paediatric COVID-19. *Lancet Infect Dis*. Epub ahead of print 15 May 2020. DOI: 10.1016/S1473-3099(20)30396-0.

16. Fridman S, Bullrich MB, Jimenez-Ruiz A, *et al.* Stroke risk, phenotypes, and death in COVID-19: systematic review and newly reported cases. *Neurology* 2020; 95: e3373–e3385.

17. Cavayas YA, Del Sorbo L and Fan E. Intracranial hemorrhage in adults on ECMO. *Perfusion* 2018; 33: 42–50.

18. AbdelRazek MA, Leone MJ and Venna N. Hemorrhagic encephalitis associated with H3N2 influenza A viral pneumonia. *Neurology* 2018; 90: 336–337.

19. Fotuhi M, Mian A, Meysami S, *et al.* Neurobiology of COVID-19. *J Alzheimers Dis* 2020; 76: 3–19.

20. Oxley TJ, Mocco J, Majidi S, *et al.* Large-vessel stroke as a presenting feature of Covid-19 in the young. *N Engl J Med* 2020; 382: e60.

21. Li Y, Li M, Wang M, *et al.* Acute cerebrovascular disease following COVID-19: a single center, retrospective, observational study. *Stroke Vasc Neurol* 2020; 382: e60.

22. Phua J, Weng L, Ling L, *et al.* Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations. *Lancet Respir Med* 2020; 8: 506–517.

23. Lechien JR, Chiesa-Estomba CM, De Siati DR, *et al.* Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur Arch Otorhinolaryngol* 2020; 277: 2251–2261.

24. Spinato G, Fabbris C, Polesel J, *et al.* Alterations in smell or taste in mildly symptomatic outpatients with SARS-CoV-2 infection. *JAMA* 2020; 323: 2089–2090.

25. Vaira LA, Salzano G, Deiana G, *et al.* Anosmia and ageusia: common findings in COVID-19 patients. *Laryngoscope* 2020; 130: 1787.

26. Asadi-Pooya AA, Yaghoubi E, Nikseresht A, *et al.* The neurological manifestations of H1N1 influenza infection; diagnostic challenges and recommendations. *Iran J Med Sci* 2011; 36: 36–39.

27. Nichols WG, Peck Campbell AJ and Boeckh M. Respiratory viruses other than influenza virus: impact and therapeutic advances. *Clin Microbiol Rev* 2008; 21: 274–290.

28. Bohmwald K, Galvez NMS, Rios M, *et al.* Neurologic alterations due to respiratory virus infections. *Front Cell Neurosci* 2018; 12: 386.

29. Bellon M, Schweblin C, Lambeng N, *et al.* Cerebrospinal fluid features in SARS-CoV-2 RT-PCR positive patients. *Clin Infect Dis*. Epub ahead of print 8 August 2020. DOI: 10.1093/cid/ciaa1165.

30. Li YC, Bai WZ and Hashikawa T. Response to commentary on "The neuroinvasive potential of SARS-CoV-2 may play a role in the respiratory failure of COVID-19 patients". *J Med Virol* 2020; 92: 707–709.

31. Moriguchi T, Harii N, Goto J, *et al.* A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int J Infect Dis* 2020; 94: 55–58.

32. Westhoff TH, Seibert FS, Bauer F, *et al.* Allograft infiltration and meningoencephalitis by SARS-CoV-2 in a pancreas-kidney transplant recipient. *Am J Transplant* 2020; 20: 3216–3220.

33. Liu JM, Tan BH, Wu S, *et al.* Evidence of central nervous system infection and neuroinvasive routes, as well as neurological involvement, in the lethality of SARS-CoV-2 infection. *J Med Virol*. Epub ahead of print 1 October 2020. DOI: 10.1002/jmv.26570.

34. Bhatraju PK, Ghassemieh BJ, Nichols M, *et al.* Covid-19 in critically ill patients in the Seattle region - case series. *N Engl J Med* 2020; 382: 2012–2022.

35. Li H, Liu SM, Yu XH, *et al.* Coronavirus disease 2019 (COVID-19): current status and future perspectives. *Int J Antimicrob Agents* 2020; 55: 105951.

36. Li MY, Li L, Zhang Y, *et al.* Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. *Infect Dis Poverty* 2020; 9: 45.

37. Xiong M, Liang X and Wei YD. Changes in blood coagulation in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. *Br J Haematol* 2020; 189: 1050–1052.

38. Minagar A and Alexander JS. Blood-brain barrier disruption in multiple sclerosis. *Mult Scler* 2003; 9: 540–549.

39. De Meyer SF, Denorme F, Langhauser F, *et al.* Thromboinflammation in stroke brain damage. *Stroke* 2016; 47: 1165–1172.

40. Gob E, Reymann S, Langhauser F, *et al.* Blocking of plasma kallikrein ameliorates stroke by reducing thromboinflammation. *Ann Neurol* 2015; 77: 784–803.

41. van Vliet EA, da Costa Araujo S, Redeker S, *et al.* Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. *Brain* 2007; 130: 521–534.

42. Sweeney MD, Sagare AP and Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol* 2018; 14: 133–150.

43. Stettner M, Lohmann B, Wolffram K, *et al.* Interleukin-17 impedes Schwann cell-mediated myelination. *J Neuroinflammation* 2014; 11: 63.

44. Spiezia L, Boscolo A, Poletto F, *et al.* COVID-19-related severe hypercoagulability in patients admitted to intensive care unit for acute respiratory failure. *Thromb Haemost* 2020; 120: 998–1000.

45. Kim JE, Heo JH, Kim HO, *et al.* Neurological complications during treatment of middle east respiratory syndrome. *J Clin Neurol* 2017; 13: 227–233.