Neurosymptoms of COVID-19: results of cerebrospinal fluid and blood biomarkers and assessment of diagnostic efficacy of risk factors

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Research Article

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Abstract

Background

Novel coronavirus disease (COVID-19) patients can exhibit acute neurosymptoms when infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), but the mechanism for the occurrence of neurosymptoms in COVID-19 patients are not yet clear. The purpose of this study was to identify potential causes of neurosymptoms in COVID-19 patients by exploring the characteristics of cerebrospinal fluid (CSF) and peripheral blood in COVID-19 patients with neurosymptoms.

Methods

A study was conducted on 40 COVID-19 patients with neurosymptoms (categorized into subgroups of encephalopathy, encephalitis, and other neurosymptoms). CSF biomarkers and serum cytokines were compared between neurosymptom subgroups and COVID-19-negative control group. Blood biomarkers were compared between neurosymptom subgroups and COVID-19-positive control group. Logistic regression analysis and receiver operating characteristic (ROC) analysis were used to detect the risk factors and evaluate the diagnostic performance of risk factors for neurosymptoms in COVID-19 patients.

Results

Compared with COVID-19-negative control, encephalopathy subgroup had significantly higher values of CSF to serum albumin ratio (QAlb) and CSF interleukin-6 (IL-6) (all \( P<0.05 \)), encephalitis subgroup had significantly higher values of CSF total protein (TP), CSF albumin (Alb), QAlb, CSF white blood cell (WBC) count, and CSF IL-6 (all \( P<0.05 \)), other neurosymptom subgroup had significantly higher CSF TP (\( P<0.05 \)). In addition, serum IL-6 in all subgroups were higher than COVID-19-negative control (\( P<0.05 \)). Compared with COVID-19-positive control, all subgroups had significantly lower serum immunoglobulin G (IgG) levels (\( P<0.05 \)), significantly higher serum complement C3 (C3) levels (\( P<0.05 \)), and no differences in serum IL-6 concentrations were found between all subgroups and COVID-19-positive control (\( P>0.05 \)). Logistic regression analysis showed the levels of serum IgG and C3 might be risk factors for neurosymptoms in COVID-19 patients. The area under the curve (AUC) of serum IgG was 0.832 (95% CI 0.727–0.909, \( P<0.0001 \)), with sensitivity of 80.00%, and specificity of 73.53%. The AUC of serum C3 was 0.768 (95% CI 0.655–0.858, \( P<0.0001 \)), with sensitivity of 70.00%, and specificity 76.47%.

Conclusion

Immunological imbalance with decreased IgG levels and increased C3 levels in circulation may be key factors in the occurrence of neurosymptoms in COVID-19 patients.

Introduction

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing novel coronavirus disease (COVID-19), can lead to respiratory and various systemic symptoms, including fever, cough, and
fatigue [1, 2]. As understanding grows, clinical findings have revealed that SARS-CoV-2 infection can directly or indirectly affect the nervous system in some patients [3–5]. It has been reported that COVID-19 patients can exhibit acute central and peripheral nervous system symptoms when infected with SARS-CoV-2 [6–8], primarily presenting as encephalopathy [9, 10], COVID-19-related encephalitis [11, 12], Guillain-Barré syndrome [13], cerebrovascular diseases, etc [14]. The mechanism for the occurrence of neurosymptoms in patients infected with SARS-CoV-2 are not yet clear. Possible mechanism may include: the virus invading the brain through retrograde transmission along the brain nerve pathway or disrupting the blood-brain barrier (BBB) [15]; viral components of SARS-CoV-2 can promote immune responses in the central nervous system [16], and cytokines storm produced by SARS-CoV-2 infection reaches the central nervous system through passive diffusion across the BBB [17]; inflammation and hypoxia leading to thrombosis [18–20]. The purpose of this study was to explore the infection status of SARS-CoV-2 nucleic acid in the cerebrospinal fluid (CSF) of COVID-19 patients with neurosymptoms, as well as the changes in BBB, CSF cytology, and CSF cytokines, and to compare whether there are differential indicators in the serum of COVID-19 patients with neurosymptoms and those without, to find the causes of neurosymptoms in COVID-19 patients and provide a basis for the prevention and treatment of neurosymptoms in COVID-19 patients.

Methods

Study population

This is a cross-sectional study that includes a neurosymptom group and three neuroasymptom control groups: COVID-19-negative control group, COVID-19-positive control group, and healthy control group. The neurosymptom group consisted of hospitalized patients who were admitted to the Third Xiangya Hospital of Central South University due to neurosymptoms after exhibiting symptoms of COVID-19 between December 20, 2022, and February 19, 2023. The onset time of neurosymptoms after the first respiratory or systemic COVID-19 symptoms was limited to 1–30 days [9]. A total of 40 COVID-19 patients with neurosymptoms were included in the study. The median age of the neurosymptom group was 51 years (interquartile range [IQR] 31–61); 52.5% of the patients were males. According to the diagnostic criteria for COVID-19 in the “Novel Coronavirus Infection Diagnosis and Treatment Protocol (Trial Version 10)” issued by the National Health Commission of China in 2023, COVID-19 can be divided into four different grades: mild, moderate, severe, and critical.

In the neurosymptom group, three subgroups were further divided, namely, encephalopathy subgroup, encephalitis subgroup, and other neurosymptom subgroup. Encephalopathy subgroup included 21 patients, defined as diffuse brain dysfunction, including changes in mental state such as decreased consciousness, cognitive impairment, and behavioral changes, without signs of acute central nervous system inflammation, such as an increase in CSF cells and/or brain MRI changes. We defined this as COVID-19-related encephalopathy [9]. Encephalitis subgroup included 12 patients, defined as patients exhibiting changes in mental state such as decreased consciousness, cognitive impairment, behavioral
changes, and an increase in CSF white blood cell (WBC) count (WBC count > 5/µL) or inflammatory brain MRI changes, which were diagnosed as encephalitis [16]. Other neurosymptom subgroup included a total of 7 cases, including 4 cases of Guillain-Barré syndrome, 2 cases of multiple sclerosis, and 1 case of non-specific inflammation of the cavernous sinus with oculomotor nerve palsy. The diagnosis of Guillain-Barré syndrome was based on the Asbury and Cornblath diagnostic criteria [21]. The diagnosis of multiple sclerosis was based on the revised 2017 McDonald criteria [22]. The patient with non-specific inflammation of the cavernous sinus and oculomotor nerve palsy presented with drooping of the left upper eyelid and blurred vision 5 days after COVID-19 infection and was admitted. Cranial MRV + MRI plain scan + enhanced DWI and DSA angiography, as well as orbital plain scan + enhanced examination, showed abnormal signals and enhancement in the left cavernous sinus. After symptomatic support treatment including nourishing nerves, fluid replacement, and anti-edema treatment, the oculomotor nerve palsy improved significantly with no recurrence to date. CSF, serum, EDTA-K2 anticoagulant, and sodium citrate anticoagulant whole blood samples were collected from COVID-19 patients with neurosymptoms.

COVID-19-negative control group consisted of leukemia patients who were not infected with SARS-CoV-2 during the same period. These patients were stable after routine chemotherapy and were undergoing diagnostic lumbar puncture and prophylactic intrathecal chemotherapy. The CSF cytology, flow cytometric analysis of CSF, and brain MRI results showed no central nervous system leukemia. There were 24 patients in COVID-19-negative control group, with median age of 39 years (IQR 25–54); 66.7% of these patients were males. CSF and serum samples were collected from the COVID-19-negative control before chemotherapy. COVID-19-positive control group consisted of hospitalized patients infected with SARS-CoV-2 during the same period who did not exhibit neurosymptoms. There were 34 patients in COVID-19-positive control group, with median age of 53 years (IQR 43–65); 44.1% of these patients were males. Serum samples were collected from COVID-19-positive control, as well as EDTA-K2 anticoagulant and sodium citrate anticoagulant whole blood samples. Healthy control group included physically healthy individuals who accepted physical examinations at the same hospital during the same period and were not infected with SARS-CoV-2. Healthy control group consisted of 50 individuals, with median age of 45 years (IQR 37–59); 40.0% of these individuals were males. Serum samples were collected from healthy control group.

The neurosymptom group and COVID-19-positive controls met the WHO COVID-19 case definitions or the diagnostic criteria for COVID-19 in the “Novel Coronavirus Infection Diagnosis and Treatment Protocol (Trial Version 10)” issued by the National Health Commission of China in 2023.

The exclusion criteria for this study were: encephalopathy caused by factors such as septicemia, toxicity, or metabolic factors; neurosymptoms caused by brain tumors or infections by other pathogens; patients with acute or chronic peripheral nerve diseases caused by factors such as diabetes or tumors; patients with relapses of neurological diseases; patients with cerebrovascular disease. Subjects who received immunoglobulins, glucocorticoids, or anticoagulation therapy before specimen collection were excluded from the study. This study complied with medical ethics requirements, was approved by the Ethics
Committee of the Third Xiangya Hospital of Central South University (approval number: Quick 23156), and all subjects voluntarily participated in the study and signed informed consent forms.

Research methods

SARS-CoV-2 nucleic acid detection: Reagents for SARS-CoV-2 nucleic acid extraction, purification, and detection were provided by Shanghai BioGerm Medical Technology Co., Ltd. (BioGerm, China). ORF1ab and N genes of SARS-CoV-2 were detected, and if positive, the test was repeated.

CSF routine chemistry and BBB markers: CSF total protein (TP), albumin (Alb), CSF glucose, CSF chloride, and CSF lactate dehydrogenase were tested using a Hitachi 7600 automatic biochemical analyzer (Hitachi, Japan). Serum Alb were tested using a Hitachi 7600 automatic biochemical analyzer. QAlb was calculated as follows: QAlb = CSF Alb/serum Alb × 1000.

Inflammatory markers: CSF and serum cytokines, including interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ), and interleukin-17A (IL-17A), were tested using BD FACSCanto II flow cytometer (BD, USA) with reagents from Tianjin Kainuo Tongsheng Biotechnology Co., Ltd. CSF and serum IL-6 were tested using Roche cobase 601 electrochemiluminescence immunoassay analyzer (Roche, Switzerland). Other indicators, including peripheral blood WBC count, lymphocyte count, and C-reactive protein (CRP), were tested using Mindray BC6800 automatic hematology analyzer (Shenzhen Mindray, China). Serum procalcitonin (PCT) was tested using Roche cobase 601 electrochemiluminescence immunoassay analyzer (Roche, Switzerland).

Coagulation function indicators: Prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimer, thrombin time (TT), fibrinogen (FIB) were tested using Sysmex CS5100 automatic coagulation analyzer (Sysmex, Japan).

Immune function indicators: Immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), complement C3 (C3), and complement C4 (C4) were tested using Beckman IMMAGE800 automatic immunoassay analyzer (Beckman, USA).

Statistical analysis

Measurement data are expressed as median (IQR). Numerical variables were log10 or log2 transformed where appropriate. Comparison between two groups was performed using the Mann-Whitney U test. Count data are expressed as cases (%), and comparisons between two groups were made using the chi-square test or Fisher exact test. Single-factor logistic regression analysis was conducted first, followed by multi-factor logistic regression analysis of the variables with \( P < 0.05 \) in the single-factor analysis to determine the risk factors for the occurrence of neurosymptoms in COVID-19 patients. Receiver operating characteristic (ROC) curves were plotted for risk factors, and the area under the curve (AUC) was calculated to evaluate the diagnostic efficiency of individual and combined risk factors for the occurrence of neurosymptoms in COVID-19 patients. The calculation and comparison of the area under the ROC
curve were performed using MedCalc software version 15.2.2, and other analyses were performed using GraphPad Prism 9.0 or SPSS 21.0. $P<0.05$ was considered statistically significant.

Results

SARS-CoV-2 nucleic acid test results

Results of SARS-CoV-2 nucleic acid test on CSF from 40 COVID-19 patients with neurosymptoms showed that in one 42-year-old patient with encephalitis, SARS-CoV-2 RNA was detected in the CSF. The CT values for ORF1ab and N genes were 36.03 and 36.33, respectively. The repeat test results were 36.26 and 36.72, respectively. CSF SARS-CoV-2 nucleic acid tests in the rest of COVID-19 patients with neurosymptoms were negative.

Comparison of CSF biomarkers

The comparison of parameters between neurosymptom subgroups and COVID-19-negative control group is as follows: gender, age, CSF TP, CSF Alb, QAlb, CSF WBC count, CSF glucose, CSF chloride, CSF lactate dehydrogenase, CSF IL-2, IL-4, IL-6, IL-10, TNF-α, INF-γ, IL-17A. Compared with COVID-19-negative control group, QAlb and CSF IL-6 in encephalopathy subgroup were significantly higher (all $P<0.05$), with no differences in other indicators (all $P>0.05$) (Table 1, Fig. 1), CSF WBC count, CSF TP, CSF Alb, QAlb, and CSF IL-6 in encephalitis subgroup were significantly higher (all $P<0.05$), with no differences in other indicators (all $P>0.05$) (Table 1, Fig. 1). CSF TP in other neurosymptom subgroup was significantly higher than COVID-19-negative control group ($P<0.05$), with no differences in other indicators (all $P>0.05$) (Table 1, Fig. 1).

Comparison of blood biomarkers

The comparison of parameters between the neurosymptom subgroups and COVID-19-negative control group includes: gender, age, serum IL-2, IL-6, IL-4, IL-10, TNF-α, INF-γ, IL-17A. Compared with COVID-19-negative control group, serum IL-6 were significantly higher in all neurosymptom subgroups ($P<0.05$), with no differences in other indicators (all $P>0.05$) (Table 1).

The comparison of parameters between the neurosymptom subgroups and COVID-19-positive control group includes: gender, age, severity of COVID-19, the time of serum sample collection after COVID-19 onset, prevalence of hypertension and other underlying diseases, serum IL-2, IL-4, IL-6, IL-10, TNF-α, INF-γ, IL-17A, serum IgA, IgG, IgM, C3, C4, TP, Alb, WBC count, lymphocyte count, CRP, PCT, PT, APTT, TT, FIB, D-dimer.

Compared with COVID-19-positive control group, FIB and IgG in encephalopathy subgroup were significantly lower (all $P<0.05$), and C3 was significantly higher (all $P<0.05$), with no differences in other indicators (all $P>0.05$) (Table 2, Fig. 2).
Compared with COVID-19-positive control group, IgG in encephalitis subgroup was significantly lower ($P<0.05$), and C3 was significantly higher ($P<0.05$), with no differences in other indicators (all $P>0.05$) (Table 2, Fig. 2).

Compared with COVID-19-positive control group, IgG in other neurosymptom subgroup was significantly lower ($P<0.05$), lymphocyte count and C3 were significantly higher (all $P<0.05$), with no differences in other indicators (all $P>0.05$) (Table 2, Fig. 2).

**Comparison of serum IgG and C3 concentrations in neurosymptom group with healthy control group**

The above results showed that serum IgG and C3 levels in encephalopathy, encephalitis, and other neurosymptom subgroup were significantly different from those in COVID-19-positive control group. Therefore, further comparison of IgG and C3 with healthy control group was conducted, and the results (Table 3) showed that IgG in neurosymptom group was significantly lower than in healthy control group, and C3 was significantly higher than in healthy control group (all $P<0.05$), while the levels of IgG and C3 in COVID-19-positive control group were not significantly different from those in healthy control group (all $P>0.05$).
Table 3
Comparison of serum immune function in neurosymptom group and COVID-19-positive control group with healthy control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Neurosymptoms (Encephalopathy + Encephalitis + Other neurosymptoms- n = 40)</th>
<th>COVID-19-positive control (n = 34)</th>
<th>Healthy control (n = 50)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neurosymptoms vs healthy control</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>21 (52.5)</td>
<td>15(44.1)</td>
<td>20(40.0)</td>
<td>0.237</td>
</tr>
<tr>
<td>Age, years</td>
<td>51(31–61)</td>
<td>53(43–65)</td>
<td>45(37–59)</td>
<td>0.852</td>
</tr>
<tr>
<td>IgA, g/L</td>
<td>1.93(1.32–2.62)</td>
<td>2.34(1.51–3.27)</td>
<td>1.90(1.56–2.95)</td>
<td>0.170</td>
</tr>
<tr>
<td>IgG, g/L</td>
<td>9.45(5.20–10.48)</td>
<td>12.95(10.07–14.43)</td>
<td>11.40(10.48–12.93)</td>
<td>0.000</td>
</tr>
<tr>
<td>IgM, g/L</td>
<td>0.97(0.74–1.37)</td>
<td>1.03(0.68–1.22)</td>
<td>1.07(0.84–1.69)</td>
<td>0.381</td>
</tr>
<tr>
<td>C3, g/L</td>
<td>1.09(0.88–1.30)</td>
<td>0.81(0.64–0.96)</td>
<td>0.84(0.77–0.95)</td>
<td>0.000</td>
</tr>
<tr>
<td>C4, g/L</td>
<td>0.24(.018-0.28)</td>
<td>0.20(0.18–0.27)</td>
<td>0.20(0.17–0.23)</td>
<td>0.150</td>
</tr>
</tbody>
</table>

Data are presented as median (IQR) unless otherwise indicated. P value were calculated using Mann-Whitney U test for continuous variables and chi-square test for categorical variables. IQR, interquartile range; COVID-19, coronavirus disease 2019; IgG, Immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; C3, complement C3; C4, complement C4

Concentrations of IgG and C3 might be independent risk factors for the occurrence of neurosymptoms in COVID-19 patients

The results of univariate logistic regression analysis (Table 4) and multivariate logistic regression analysis (Table 5) indicated that the levels of serum IgG and C3 might be independent risk factors for the occurrence of neurosymptoms in COVID-19 patients.
### Table 4
The results of univariate logistic regression analysis of risk factors for COVID-19 patients with neurosymptoms

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1.400 (0.559–3.507)</td>
<td>0.473</td>
</tr>
<tr>
<td>Age</td>
<td>1.018 (0.993–1.043)</td>
<td>0.160</td>
</tr>
<tr>
<td>Time from COVID-19 onset to blood sampling</td>
<td>1.009 (0.943–1.080)</td>
<td>0.791</td>
</tr>
<tr>
<td>Severity of COVID-19</td>
<td>0.944 (0.481–1.854)</td>
<td>0.867</td>
</tr>
<tr>
<td>Basic diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.361 (0.756–7.376)</td>
<td>0.139</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>1.194 (0.225–6.340)</td>
<td>0.835</td>
</tr>
<tr>
<td>Heart failure</td>
<td>0.576 (0.05–6.641)</td>
<td>0.658</td>
</tr>
<tr>
<td>Arteriosclerosis</td>
<td>0.629 (0.167–2.363)</td>
<td>0.429</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>1.644 (0.341–7.924)</td>
<td>0.535</td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>0.548 (0.126–2.382)</td>
<td>0.423</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.977 (0.270–3.535)</td>
<td>0.972</td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>1.187 (0.158–8.912)</td>
<td>0.867</td>
</tr>
<tr>
<td>Otitis media/mastoiditis</td>
<td>0.273 (0.029–2.566)</td>
<td>0.256</td>
</tr>
<tr>
<td>Blood biomarkers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>0.891 (0.685–1.159)</td>
<td>0.389</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.843 (0.627–1.133)</td>
<td>0.258</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.044 (0.993–1.016)</td>
<td>0.459</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.970 (0.848–1.108)</td>
<td>0.650</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.844 (0.594–1.199)</td>
<td>0.344</td>
</tr>
<tr>
<td>INF-γ</td>
<td>1.120 (0.694–1.799)</td>
<td>0.640</td>
</tr>
<tr>
<td>IL-17A</td>
<td>1.121 (0.736–1.707)</td>
<td>0.595</td>
</tr>
<tr>
<td>IgA</td>
<td>1.445 (0.925–2.259)</td>
<td>0.106</td>
</tr>
</tbody>
</table>

COVID-19, coronavirus disease 2019; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor-α; INF-γ, interferon-γ; IL-17A, interleukin-17A; IgG, Immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; C3, complement C3; C4, complement C4; WBC, white blood cell; CRP, C-reactive protein; PCT, procalcitonin; PT, prothrombin time, APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen; TP, total protein; Alb, albumin
<table>
<thead>
<tr>
<th>Parameters</th>
<th>OR(95%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>1.665(1.292–2.146)</td>
<td>0.000</td>
</tr>
<tr>
<td>IgM</td>
<td>0.842(0.522–1.358)</td>
<td>0.480</td>
</tr>
<tr>
<td>C3</td>
<td>0.027(0.003–0.223)</td>
<td>0.001</td>
</tr>
<tr>
<td>C4</td>
<td>0.075(0.000–14.548)</td>
<td>0.336</td>
</tr>
<tr>
<td>WBC</td>
<td>1.001(0.917–1.092)</td>
<td>0.985</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>0.584(0.282–1.210)</td>
<td>0.148</td>
</tr>
<tr>
<td>CRP</td>
<td>1.004(0.998–1.010)</td>
<td>0.188</td>
</tr>
<tr>
<td>PCT</td>
<td>1.057 (0.818–1.366)</td>
<td>0.670</td>
</tr>
<tr>
<td>PT</td>
<td>0.996(0.724–1.369)</td>
<td>0.979</td>
</tr>
<tr>
<td>APTT</td>
<td>1.006(0.932–1.087)</td>
<td>0.872</td>
</tr>
<tr>
<td>TT</td>
<td>0.948(0.799–1.125)</td>
<td>0.542</td>
</tr>
<tr>
<td>FIB</td>
<td>1.401(0.918–2.138)</td>
<td>0.118</td>
</tr>
<tr>
<td>D-dimer</td>
<td>1.051(0.920–1.200)</td>
<td>0.464</td>
</tr>
<tr>
<td>TP</td>
<td>1.031(0.966–1.099)</td>
<td>0.361</td>
</tr>
<tr>
<td>ALB</td>
<td>0.960(0.877–1.052)</td>
<td>0.384</td>
</tr>
</tbody>
</table>

COVID-19, coronavirus disease 2019; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor-α; INF-γ, interferon-γ; IL-17A, interleukin-17A; IgG, Immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; C3, complement C3; C4, complement C4; WBC, white blood cell; CRP, C-reactive protein, PCT, procalcitonin; PT, prothrombin time, APTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen; TP, total protein; Alb, albumin

Table 5
The results of multivariate logistic regression analysis of risk factors for COVID-19 patients with neurosymptoms

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OR(95%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.694(0.173–2.789)</td>
<td>0.607</td>
</tr>
<tr>
<td>Age</td>
<td>1.029(0.990–1.069)</td>
<td>0.148</td>
</tr>
<tr>
<td>Time from COVID-19 onset to blood sampling</td>
<td>1.067(0.956–1.190)</td>
<td>0.248</td>
</tr>
<tr>
<td>IgG</td>
<td>1.795(1.316–2.448)</td>
<td>0.000</td>
</tr>
<tr>
<td>C3</td>
<td>0.021(0.001–0.304)</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Efficacy analysis of serum IgG, C3, and IgG combined with C3 in diagnosing neurosymptoms in COVID-19 patients

With the occurrence of neurosymptoms in 74 COVID-19 patients as the dependent variable, and serum IgG, C3, and IgG combined with C3 as the detection variables, ROC curves were drawn (Fig. 3). The area under the curve (AUC) of serum IgG for diagnosing COVID-19 patients with neurosymptoms was 0.832 (95% CI 0.727–0.909, \( P < 0.0001 \)), with optimal cutoff value of 10.5 g/L, sensitivity of 80.00%, and specificity of 73.53%. The AUC of serum C3 for diagnosing COVID-19 patients with neurosymptoms was 0.768 (95% CI 0.655–0.858, \( P < 0.0001 \)), with optimal cutoff value of 0.96 g/L, sensitivity of 70.00%, and specificity of 76.47%. The AUC of IgG combined with C3 for diagnosing COVID-19 patients with neurosymptoms were 0.890 (95% CI 0.796–0.951, \( P < 0.0001 \)), with optimal cutoff value of 0.4432, sensitivity of 82.50%, and specificity of 88.24%. There was no significant difference in the AUC between C3 and IgG (\( Z = 0.863, P = 0.3883 \)). The AUC for C3 in combination with IgG was significantly different compared to C3 alone (\( Z = 2.607, P = 0.0091 \)), while there was no significant difference in the AUC between IgG and IgG in combination with C3 (\( Z = 1.656, P = 0.0977 \)). Therefore, IgG combined with C3 has higher diagnostic efficacy than single C3 in diagnosing the occurrence of neurosymptoms in COVID-19 patients.

Discussion

This study is a cross-sectional study that classifies COVID-19 patients with neurosymptoms. In this study, the largest number of COVID-19 neurosymptoms patients had combined encephalopathy, followed by patients with encephalitis and other neurosymptoms. This study found that among the 40 COVID-19 neurosymptoms patients, only 1 case had detectable SARS-CoV-2 nucleic acid in CSF, similar to previous research results [16, 23], indicating that direct invasion of the brain by SARS-CoV-2 resulting in neurosymptoms is rare.

This study found that QAlb in both encephalopathy and encephalitis subgroups was significantly increased, consistent with previous research results [24, 25]. QAlb is an indicator reflecting BBB damage. Under normal circumstances, microvascular endothelial cells of BBB protect the central nervous system from toxins and microorganisms in blood. These cells express tight junction proteins that restrict movement between adjacent cells and control the entry and exit of molecules from the blood to the brain parenchyma through specific transport proteins and receptor proteins [26]. The infection of SARS-CoV-2 may affect the integrity of BBB through different mechanisms, such as direct interaction of SARS-CoV-2 with BBB components, coagulation disorders, hypoxia, and inflammatory responses, which may play important roles in inducing BBB damage [15]. Further research is needed to investigate the mechanisms for BBB damage caused by SARS-CoV-2.

Currently, it is controversial whether there were increased inflammatory factors in CSF of COVID-19 patients with neurosymptoms [9, 17, 27–30]. These results of this study showed that the levels of IL-6 in CSF of encephalopathy and encephalitis subgroups were significantly higher than in COVID-19-negative
control group. However, the levels of IL-6 and other cytokines in other neurosymptoms subgroup showed no significant difference compared to COVID-19-negative control group. These indicates that the increase of cytokines in CSF is not consistent among patients with different neurosymptoms.

To further investigate whether the increased IL-6 in CSF of encephalopathy and encephalitis subgroup were derived from cytokines storm in peripheral blood after SARS-CoV-2 infection, we compared the cytokines in peripheral blood of each subgroup with those of COVID-19-negative control group and COVID-19-positive control group. We found that IL-6 levels in all subgroups were higher than in COVID-19-negative control group. However, there was no statistical difference in peripheral blood IL-6 concentration when comparing with COVID-19-positive control group. Therefore, the authors speculated that although COVID-19 patients have a systemic inflammatory response, the increased cytokines in peripheral blood are not enough to cause neurosymptoms in COVID-19 patients. After comparing other indicators of three neurosymptom subgroups and COVID-19-positive control group, the authors found that serum IgG levels were significantly lower and C3 levels were significantly higher in three subgroups compared to COVID-19-positive control group. Moreover, IgG levels in neurosymptom group were significantly lower than in healthy control group, while C3 levels were significantly higher than in healthy control group. Subsequently, univariate and multivariate logistic regression analysis of serum IgG and C3 showed that the differences in serum IgG and C3 levels might be independent risk factors for COVID-19 patients with neurosymptoms.

It has been demonstrated that the binding of the S protein of SARS-CoV-2 can trigger 83 unique genes in human brain endothelial cells, upregulating C3 [31]. When C3 was cleaved into C3a and C3b, the C3a fragment acts as an effective cytokine amplifying the inflammatory signal, and C3b binding to other complement factors on the cell surface can lead to cell destruction [32]. It has been reported that in some neurological diseases or autoimmune diseases, patients with severe disease and those in the active stage have higher levels of C3 in serum. Increased levels of C3 in serum were associated with adverse outcomes [33–35], and researchers have also found that C3 activation is a key component of complement-related inflammatory tissue damage after stroke [36]. Circulating C3 contributes to the activation of brain endothelial cells, the complement system plays an important role in central nervous system inflammatory responses [37]. These evidences suggest that the increased C3 in circulation may be an important cause of BBB damage and COVID-19 patients with neurosymptoms. [32].

IgG is the most abundant class in serum immunoglobulins, and the basic structure of the IgG molecule is composed of peptide chains, forming a Y-shaped structure with two identical heavy chains and two identical light chains. Digestion of papain can produce two antigen-binding fragments (Fab) and one crystallizable fragment (Fc). On the one hand, they induce proinflammatory reactions and recruit innate immune effector cells during pathogenic microbial infection or autoimmune diseases [38]. On the other hand, they promote phagocytosis by binding to Fc receptors, clear immune complexes in body, and can reduce Fc receptor and complement-dependent inflammatory responses [38–40]. A decrease in its level may significantly reduce its anti-inflammatory and immunomodulatory effects, promoting the occurrence and development of diseases [39, 41, 42]. Serum IgG plays an important role in monitoring many
diseases, and Wang Zheng et al. study on patients with ulcerative colitis found that the proportion of severe patients was significantly higher in patients with low blood IgG levels compared to patients with normal IgG levels. Serum IgG is a valuable indicator for predicting the severity of ulcerative colitis [39]. In addition, the serum IgG of deceased patients with sepsis was significantly lower than that of survived patients [43]. Intravenous immunoglobulin therapy has been used for neurological diseases related to COVID-19, including encephalopathy, encephalitis, Guillain-Barré syndrome, and status epilepticus. The results showed an improvement in these patients' symptoms [44–46], and intravenously administered immunoglobulins mainly consist of IgG (accounting for about 95%) [47]. It has been reported that exogenous IgG can deactivate complement [40]. Therefore, the authors inferred that the decrease in circulating IgG may also be an important factor in occurrence of neurosymptoms in COVID-19 patients.

Furthermore, this study also found that there were other heterogeneities in the clinical spectrum and peripheral blood indicators of patients in different subgroups. For example, serum FIB in encephalopathy subgroup was significantly lower than in COVID-19-positive control group, peripheral blood lymphocyte count was significantly higher in other neurosymptom subgroup than in COVID-19-positive control group. However, the coagulation function indicators (except FIB), PCT, and other bacterial infection indicators in three subgroups showed no significant difference compared to COVID-19-positive control group. Therefore, the authors speculate that coagulation dysfunction and concurrent bacterial infection may not be risk factors for developing neurological diseases such as encephalopathy and encephalitis after SARS-CoV-2 infection.

This study has some limitations. First, the number of samples included in the study is small, and there is no large-scale multicenter study. Second, due to the difficulty in obtaining CSF samples, the CSF samples in COVID-19-negative controls did not come from CSF samples of healthy individuals, which may have some impact on the result analysis. Third, this study is not a prospective study and did not evaluate the diagnostic efficiency of serum IgG and C3 levels in predicting COVID-19 patients with neurosymptoms. Fourth, this study found that COVID-19 patients with neurosymptoms had a decrease in IgG levels and an increase in C3 levels, but the mechanism behind this phenomenon needs further exploration. In summary, this is a new discovery, and it is hoped that it will bring new ideas to the clinical treatment and medical research of COVID-19-related neurological diseases.

**Conclusion**

These results of this study suggest that the increased inflammatory cytokines storm with increased IL-6 may be the basis for the occurrence of neurosymptoms in COVID-19 patients. Immune imbalance with decreased circulating IgG levels and increased C3 levels may be key factors for the occurrence of neurosymptoms in COVID-19 patients. ROC curve analysis of all COVID-19 patients included in the study showed that serum IgG, C3 and their combined variables had high diagnostic efficacy. When serum IgG level in COVID-19 patients is below 10.5 g/L or serum C3 level is above 0.96 g/L, corresponding clinical measures may be needed to prevent or correct neurosymptoms in COVID-19 patients.
Abbreviations

COVID-19  Coronavirus disease 2019
SARS-CoV-2  Respiratory syndrome coronavirus 2
BBB  Blood-brain barrier
IQR  Interquartile range
CSF  Cerebrospinal fluid
WBC  White blood cell;
Alb  Albumin
TP  Total protein
QAlb  Cerebrospinal fluid to serum albumin ratio
IL-2  Interleukin-2
IL-4  Interleukin-4
IL-6  Interleukin-6
IL-10  Interleukin-10
TNF-α  Tumor necrosis factor-α
INF-γ  Interferon-γ
IL-17A  Interleukin-17A
IgG  Immunoglobulin G
IgA  Immunoglobulin A
IgM  Immunoglobulin M
C3  Complement C3
C4  Complement C4
CRP  C-reactive protein
PCT  Procalcitonin
PT Prothrombin time
APTT Activated partial thromboplastin time
TT Thrombin time
FIB Fibrinogen

Declarations

Author contributions
HC and CP designed this study and drafted the original manuscript. JT and HZ revised the manuscript. KS, XT, HX performed the laboratory analysis, HC and CP and QL analyzed the data and performed statistical analysis. All authors contributed to the article and approved the submitted version.

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Availability of data and materials
All the data supporting the findings of this study are available on request from the corresponding author.

Ethics approval and consent to participate
This study complied with medical ethics requirements, was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University (approval number: Quick 23156), and all subjects voluntarily participated in the study and signed informed consent forms.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

References


Compared With Control Participants Without Infection or Neurologic Symptoms. JAMA Netw Open. 2022;5(5):e2213253.


Tables

Tables 1 and 2 are available in the Supplementary Files section.

Figures

Figure 1

Values of CSF TP, CSF Alb, QAlb in neurosymptom sugroups and COVID-19-negative control, boxes indicate the median and interquartile range (IQR), whiskers indicate maximum and minimum values. Serum IL-6 were log10 transformed, CSF Alb, QAlb were log2 transformed. Comparative analyses were performed using Mann-Whitney U test. COVID-19, coronavirus disease 2019; Alb, albumin; QAlb, CSF to serum albumin ratio; CSF, Cerebrospinal fluid; TP, total protein. (*) p<0.05; (**) p<0.01; (****) p<0.0001; ns, not significant.
Figure 2

Serum IL-6, IgG, C3 concentrations in neurosymptom subgroups and COVID-19-positive control, boxes indicate the median and interquartile range (IQR), whiskers indicate maximum and minimum values. Serum IL-6 were log10 transformed. Comparative analyses were performed using Mann-Whitney U test. COVID-19, coronavirus disease 2019; IL-6, interleukin-6; IgG, Immunoglobulin G; C3, complement C3. (*) p<0.05; (**) p<0.01; (****) p<0.0001; ns, not significant.
**Figure 3**

Receiver operating characteristic (ROC) curves of IgG, C3 and IgG in combination with C3 for diagnosing of COVID-19 patients with neurosymptoms.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)
- [Table2.xlsx](#)