Short Communication

Brain stem encephalitis is a rare complication of COVID-19

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ABSTRACT

Here, we describe the clinical phenotype of SARS-CoV-2-related CNS disease and evaluate the SARS-CoV-2 antibody index as a tool to differentiate between a direct (viral) and indirect etiology. Out of >4000 hospitalized patients with COVID-19, we included 13 patients with neurological symptoms with suspicion of neuroinflammation. On clinical grounds, eight were classified as having a possible/probable relationship between neurological symptoms and COVID-19. A clinically distinctive phenotype of brainstem and cerebellar symptoms was seen in 6/8 patients. As we found a positive SARS-CoV-2 antibody index in 3/5 patients, indicating specific intrathecal SARS-CoV-2 IgG production, a direct link with SARS-CoV-2 is likely.

1. Introduction

Increasing numbers of reports support an association between SARS-CoV-2 infection and neurological symptoms. In hospitalized patients with COVID-19, neurological symptoms occur in 8.8–57.4% (Mao et al., 2020; Romero-Sanchez et al., 2020; Meppiel et al., 2021). These manifestations vary from nonspecific symptoms such as myalgia and headache, to encephalopathy, stroke and encephalitis (Meppiel et al., 2021). This broad variety of syndromes led to the hypothesis of a multifactorial pathogenesis, involving either direct viral neuroinvasion, an immune-mediated mechanism (i.e. SARS-CoV-2-induced autoimmune encephalitis) or indirect effects (e.g. metabolic disturbances, coagulopathy) (Ellul et al., 2020).

Since SARS-CoV-2 genome is rarely detected in CSF of patients with COVID-19 and neurological symptoms, other diagnostic tools are required to distinguish between etiologies (Lewis et al., 2021; Ellul et al., 2020). Antibody Index serology is a tool to demonstrate local antibody production in immune-privileged sites such as the central nervous system (CNS) (Shamier et al., 2021). In viral encephalitis, either a positive molecular test (i.e. PCR, detection of SARS-CoV-2 genome) on CSF or the presence of a specific intrathecal antibody response is considered as confirmatory for an etiological diagnosis (Granerod et al., 2010).

The aim of this study was to describe the clinical neurological phenotypes associated with COVID-19, with exclusion of those related to vascular complications, and to evaluate the SARS-CoV-2 antibody index as a potential tool to differentiate between SARS-CoV-2-related inflammatory disease of the CNS and other (indirect) causes of neurological disease.

2. Methods

2.1. Study population

The Erasmus Medical Center is a tertiary care hospital in Rotterdam,
the Netherlands, and is a national reference center for both clinical virology and neuroinflammatory diseases. Hospitalized COVID-19 cases with neurological symptoms in whom a COVID-19-related neuroinflammatory cause was suspected and who were discussed with one of our neuroimmunologists were included in this study. Patients with vascular complications related to COVID-19 were excluded. Based on clinical information (symptoms, routine laboratory tests, brain imaging, CSF analysis and autoimmune antibody testing) alternative explanations were ruled out and the likelihood of a SARS-CoV-2 associated etiology was assessed by a neuropathologist. To exclude non-COVID-19 viral etiologies, CSF was tested for the presence of viral genome of common neurotropic viruses (including herpes simplex virus (HSV), varicella zoster virus (VZV) and enterovirus). Cases with viral encephalitis caused by viruses other than SARS-CoV-2 were excluded. The relationship between COVID-19 and neurological symptoms was described as probable, possible, unlikely or postinfectious (Supplementary Table 1). Cases classified as unlikely were considered controls. Furthermore, 4 deceased COVID-19 patients without neurological symptoms, of whom CSF and serum samples were collected post-mortem, were assigned to the control group. These patients died because of progressive respiratory failure due to COVID-19.

2.2. Antibody index calculation

IgG antibody titers against SARS-CoV-2 were measured in paired serum and CSF using an in-house quantitative immunofluorescence assay. Antibody indices were calculated as previously described (Reiber and Peter, 2001). In short, multi-spot slides were coated with 20 μL VeroE6 cell suspension per spot, with a minimum cell density of 80%. Subsequently, the cells were infected with 20 μL of 1:100 SARS-CoV-2 stock per spot, followed by a 7–8 h incubation at 37 °C and a 20 min fixation. Serum dilutions were incubated on the slides and after washing the slides were stained with a conjugated anti-human immunoglobulin. To correct for potential blood-CSF barrier dysfunction and polyclonal intrathecal IgG production, albumin and IgG were measured in serum and CSF by nephelometry. To rule out antibody index positivity due to polyclonal B-cell activation, antibody indices were simultaneously calculated for HSV. As previously described, the interpretation of antibody indexes requires specific laboratory expertise. Following validation studies, values above 3 in CSF were interpreted as strong evidence for intrathecal antibody production, values between 1.5 and 3 were interpreted with caution as the risk of false-positives is larger in this range (Shamier et al., 2021).

2.3. Autoimmune antibody testing

Serum and CSF samples were tested for autoimmune antibodies. Samples were tested extensively for specific antibodies by cell-based assays (CBAs), by ELISA for GAD65 antibodies and screened for extracellular antibodies using immunohistochemistry (IHC) (De Bruijn et al., 2021).

2.4. Medical ethical approval

Our patients were included according to IRB approved studies (METC-2015-306 and METC-2020-0418). All samples and data used in this study were collected in the context of routine clinical care. Informed consent for the deceased patients was waived by the institutional privacy knowledge office.

3. Results

Over 4000 COVID-19 cases were hospitalized between April 2020 and August 2021. Thirteen patients with neurological symptoms considered potentially infectious or inflammatory, who were discussed with one of our neuroimmunologists, were included. Based on clinical information, 8 of these cases were classified as having a possible or probable relationship between neurological symptoms and COVID-19. One patient was classified as post-infectious (Table 1). Four patients, with an unlikely relationship between neurological symptoms and COVID-19, were assigned to the control group (Supplementary Table 2). In these cases, neurological symptoms were secondary to metabolic disturbances. In none of the patients SARS-CoV-2 RNA was demonstrated in CSF by RT-PCR (tested in 10/13 patients with sufficient CSF volume).

Eight patients with a probable (n = 7) or possible (n = 1) relationship had a median age of 64 years (range 50–87). Neurological manifestations included mainly myoclonia (6/8), dysarthria (5/8), ataxia (4/8), eye movement disorders (3/8) and cognitive disorders (3/8; only mild). These primarily brainstem and cerebellar symptoms were seen in 6 patients. The other 2 patients (case 3 and 8) had symptoms consistent with myelitis and mes- and diencephalitis respectively. The median time between a positive SARS-CoV-2 PCR and neurological symptoms was 13 days (range – 2–41). In all 8 patients, there were no signs of autoimmune encephalitis. In one patient in her early 50s with cerebellar symptoms (case 9), GAD65 antibodies were found (serum 16,300 IU/mL; CSF 50 IU/mL), regarded as post-infectious.

SARS-CoV-2 and HSV antibody indices are visualized in Fig. 1. 3/5 tested patients classified as having a probable relationship between neurological symptoms and COVID-19, had high SARS-CoV-2 antibody indices (21.83, 8.75 and 5.92), indicating intrathecal antibody synthesis. One of these patients also showed a mild intrathecal anti-HSV response, albeit much less marked than the anti-SARS-CoV-2 response (3.53 vs 8.75, respectively). The SARS-CoV-2 antibody index was 0.3 in the post-infectious GAD65-associated patient.

4. Discussion

Out of thousands of hospitalized COVID-19 patients, only 8 patients were suspected to have CNS symptoms directly related to COVID-19. Therefore we conclude that encephalitis and myelitis due to COVID-19 are very rare. However, we could identify a subgroup with a specific clinical phenotype in 6/8 patients. This phenotype included myoclonia, ataxia, dysarthria, and eye movement disorders, with only minor cognitive disorders if present, and was classified as brain stem encephalitis with cerebellar involvement. The other 2 patients also had predominantly infratentorial symptoms. Intrathecal antibody synthesis specific to SARS-CoV2 could be confirmed in 3/5 patients classified as probable. Together with the uniformity in clinical findings and the lack of an alternative explanation, a SARS-CoV-2-related etiology was considered likely in these 3 patients.

SARS-CoV-2 PCR in CSF was negative in all tested patients, and CSF pleocytosis was absent in all but one case. Proving causality between CNS symptoms and SARS-CoV-2 infection by detection of viral genome or regular CSF analysis is challenging. In situations where the utility of molecular testing is limited, such as viral infections characterized by rapid viral clearance or limited extracellular virion release, specific intrathecal antibody responses can provide diagnostic confirmation (Shamier et al., 2021; Granerod et al., 2010). In our study, the SARS-CoV-2 antibody indices correlated well with the clinical classification. In 3 other studies, with approximately 150 COVID-19 patients with neurological symptoms, intrathecal antibody synthesis was only found in 1 COVID-19 case that presented agitated confusion (Alexopoulos et al., 2020; Fleischer et al., 2021; Bellon et al., 2020). These studies included cases with a wide variety of neurological symptoms, including headache and encephalopathy. Our data emphasize that true neuroCOVID is rare, but testing for intrathecal antibody production is useful in selected cases.

For COVID-19-associated neurological disease, it remains unclear whether intrathecal anti-SARS-CoV-2 antibody production is a response to viral invasion of the CNS, or whether the neuro-inflammatory process is immune-mediated and an indirect consequence of infection (Solomon,
### Table 1
Characteristics of COVID-19 patients with neurological symptoms.

<table>
<thead>
<tr>
<th>Case ID</th>
<th>Gender, age, PMH (IA)</th>
<th>Neurological symptoms</th>
<th>Clinical Syndrome</th>
<th>Time COVID-19 diagnosis to neurological symptoms (days)</th>
<th>Imaging findings</th>
<th>CSF (cell count, protein level, glucose, OCB, BBBD)</th>
<th>Autoimmune antibodies (IHC and specific CBA’s in serum and CSF)</th>
<th>Likelihood of SARS-COV-2 related etiology based on clinical information</th>
<th>SARS-CoV-2 antibody index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male, 54y, OSAS, asthmatic bronchitis</td>
<td>Cerebellar ataxia, dysthria, myoclonia, hyperekplexia, autonomic disorder (transpiration), dysphagia, cognitive disorder (mild; language and memory, MoCA 26/30)</td>
<td>Brain stem encephalitis with cerebellar involvement and PERM</td>
<td>41</td>
<td>MRI-brain: aspecific</td>
<td>Normal (0 × 10³ cells/L, protein 0.59, glucose 5.3, no OCB, BBBD unknown)</td>
<td>Negative</td>
<td>Probable 21.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Male, 64y</td>
<td>Cerebellar ataxia, dysthria, myoclonia, eye movement disorder (ocular bobbing), cognitive disorder (mild; language and memory)</td>
<td>Brain stem encephalitis with cerebellar involvement</td>
<td>19</td>
<td>CT- and MRI-brain: normal</td>
<td>Normal (1 × 10⁶ cells/L, protein 0.34, glucose 4.4, no OCB, BBBD +)</td>
<td>Negative</td>
<td>Probable 8.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Male, 50y, renal cell carcinoma and lung emboli 3 yr before</td>
<td>Paralysis and dysesthesia legs (clinical sensory level: Th10)</td>
<td>Myelitis</td>
<td>7</td>
<td>MRI-myelum: normal</td>
<td>Pleocytosis (65 × 10³ cells/L, all mononuclear, protein 0.41, glucose 3.3, OCB unknown, no BBBD)</td>
<td>Negative</td>
<td>Probable 5.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Male, 64y</td>
<td>Cerebellar ataxia, dysthria, myoclonia, eye movement disorder (saccades), behavioural change (mild), possibly seizure cerebellar ataxia,</td>
<td>Brain stem encephalitis with cerebellar involvement</td>
<td>-²</td>
<td>CT- and MRI-brain: normal</td>
<td>Negative Probable</td>
<td>Negative Probable 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Male, 83y</td>
<td>Cerebellar ataxia, dysthria, myoclonia, eye movement disorder, cognitive disorder (mild; language)</td>
<td>Brain stem encephalitis with cerebellar involvement</td>
<td>12</td>
<td>CT- and MRI-brain: normal</td>
<td>Protein elevated (&lt;5 × 10⁵ cells/L, protein 0.79, glucose 3.6, no OCB, BBBD unknown)</td>
<td>Negative</td>
<td>Probable N/A</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Male, 87y</td>
<td>Dysarthria, myoclonia</td>
<td>Brain stem encephalitis</td>
<td>14</td>
<td>CT- and MRI-brain: aspecific</td>
<td>Normal (0 × 10⁶ cells/L, protein 0.46, glucose 3.6, OCB unknown, BBBD unknown)</td>
<td>Negative</td>
<td>Probable N/A</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Male, 75y, mitral valve insufficiency</td>
<td>Myoclonia</td>
<td>Central, no further localization possible</td>
<td>12</td>
<td>MRI-brain: normal</td>
<td>OCB + (3 × 10⁶ cells/L, protein 0.42, glucose 4.0, OCB +, no BBBD)</td>
<td>Negative</td>
<td>Probable 2.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Male, 51y</td>
<td>Paroxysmal autonomic storms with dystonia</td>
<td>Mes- and diencephalitis</td>
<td>25</td>
<td>MRI-brain: normal</td>
<td>Normal (&lt;4 × 10⁶ cells/L, protein 0.34, glucose 4.0, OCB unknown, no BBBD)</td>
<td>Negative</td>
<td>Possible 1.7</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Female, 51y</td>
<td>Cerebellar ataxia, eye movement disorder (overshoot, saccades), tremor, hyperreflexia, hyperekplexia, cognitive disorder (mild; multiple domains, MoCA 25/30)</td>
<td>Cerebellitis and PERM</td>
<td>14</td>
<td>MRI-brain: aspecific</td>
<td>Normal (&lt;5 × 10⁶ cells/L, protein 0.56, glucose 3.2, OCB unknown, BBBD+)</td>
<td>Anti-GAD65 positive in serum (titer 16.300) and CSF (titer 50), IHC positive</td>
<td>Post-infectious 0.3</td>
<td></td>
</tr>
</tbody>
</table>


³ CSF reference values: cells: <5 × 10³ per liter, protein level: 0.18–0.58 g/L, glucose level: 2.5–3.7 mmol/L, OCB: negative.

⁴ OCB were tested in 5/9 patients (case ID 1, 2, 4, 5 and 7).

⁵ Onset of neurological symptom onset was two days before development of respiratory symptoms and before SARS-CoV-2 PCR in serum was positive.

⁶ Identical bands in serum and CSF. There was no clinical and serological evidence of systemic disease.
The 8 patients with suspected COVID-19 related neurological problems all had predominantly infratentorial symptoms, mainly localized in brain stem and cerebellum. Cortical involvement was not seen. Similar symptoms were mentioned in earlier case reports and case series, describing patients with para- and post-infectious myoclonus, ataxia or opsoclonus associated with SARS-CoV-2 (Emamikah et al., 2021; Nelson et al., 2022; Sanguinetti and Ramdhani, 2021; Ishaq et al., 2021; Shah and Desai, 2021; Chan et al., 2021). These findings are in line with two autopsy studies that confirmed the neuroinvasive potential of SARS-CoV-2 (Meinhardt et al., 2020; Matschke et al., 2020). Indeed, virus could be detected in brain stem and cerebellum. Inflammatory changes with presence of cytotoxic T-lymphocytes in the brain stem were a common finding. Thus far, it remains unclear through what pathway the virus finds access to the central nervous system.

Auto-immune antibody testing in the workup, could help to distinguish between a direct and indirect viral effect. This is illustrated by case 9; a patient with cerebellar symptoms and GAD65 antibodies. Symptoms correspond to the typical COVID-19 related neurologic symptoms. However, GAD65 antibodies were present and the SARS-CoV-2 antibody index was not elevated. Whether GAD65 antibodies, and in what concentrations, were already present pre-COVID-19 remains speculative, but the clinical symptoms may have been triggered by SARS-CoV-2, therefore considered post-infectious. Post-infectious autoimmune encephalitis is rarely described in literature. In previously described patients with myoclonus, ataxia or opsoclonus, no autoimmune or paraneoplastic antibodies were found (Emamikah et al., 2021; Nelson et al., 2022; Sanguinetti and Ramdhani, 2021; Ishaq et al., 2021; Shah and Desai, 2021; Chan et al., 2021), except for one patient with antibodies directed against Purkinje cells, striatal neurons, and hippocampal neurons in both serum and CSF (Grimaldi et al., 2020). In an American cohort of >10,000 patients with COVID-19, only 5 patients met the diagnostic criteria for autoimmune encephalitis, no autoimmune antibodies were found (Sanchez et al., 2021). In Belgium, 1 patient with COVID-19 and limbic encephalitis harbored anti-Caspr2 antibodies, which was considered para-infectious (Guilmot et al., 2021). These studies did not assess SARS-CoV-2 antibody indices.

Antibody index calculations require specific and quantitative serological tools, experienced laboratory technicians, but also specific expertise for interpretation. In some cases, a positive antibody index is due to polyspecific B-cell activation in the CNS (associated with autoimmunity) in absence of infection. We included a HSV antibody index in the analysis, to account for this. Furthermore, the humoral response has an intrinsic delay, which limits the utility of an antibody index in acute cases. In this study, in one case classified as probably COVID-19-related (case 4) CSF was collected the day that COVID-19 symptoms occurred. Intrathecal antibody production was not detected, possibly because this sample was collected too early in the course of disease.

Our study has some limitations. The sample size was small. Inclusion of patients was not systematic, since possibly not all suitable cases were discussed with one of our neuroimmunologists. Cases were more likely to be included if neurological symptoms were severe or lasted longer. Although limiting sample size, stringent selection helped us clarify the common clinical phenotype useful for proof of principle. Finally, in clinical routine, collected CSF sample volumes are frequently small, limiting the number of tests that can be performed. For this reason, limited molecular tests could be performed on the CSF samples in this study and antibody index calculations could not be performed for 2 cases.

5. Conclusions

Central nervous system involvement in COVID-19 is rare, but in a small subset a clinically distinctive phenotype of brainstem encephalitis with cerebellar involvement is found. A direct link with SARS-CoV-2 is likely in this group as increased antibody indices indicate specific intrathecal SARS-CoV-2 IgG production. Proving causality between CNS symptoms and SARS-CoV-2 infection is challenging. In select cases the SARS-CoV-2 antibody index can be a useful diagnostic tool to differentiate between etiologies.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2022.578007.

References


