Usefulness of antibody index assessment in cerebrospinal fluid from patients negative for total-IgG oligoclonal bands

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Abstract

**Background:** Testing for cerebrospinal fluid (CSF)-restricted oligoclonal bands (OCB) by isoelectric focusing is used to detect intrathecally produced total IgG. By contrast, antibody indices (AI) are assessed to test for intrathecally produced antigen-specific IgG. A number of previous cases reports have suggested that AI testing might be more sensitive than OCB testing in detecting intrathecal IgG synthesis.

**Findings:** Here we report on 21 patients with positive AI for either herpes simplex virus, varicella zoster virus, cytomegalovirus, measles virus, rubella virus, or Borrelia burgdorferi in the absence of total-IgG OCB and, accordingly, in the presence of a normal total-IgG CSF/serum ratio.

**Conclusion:** Our findings indicate that AI testing should not generally be omitted in OCB-negative patients and provide a rationale for systematic and prospective studies on the comparative sensitivity and specificity of AI and total-IgG OCB testing in infectious and other diseases of the CNS.

**Keywords:** Intrathecal IgG synthesis, Antibody index, Cerebrospinal fluid, Oligoclonal bands, Herpes simplex virus, Cytomegalovirus, Varicella zoster virus, Measles virus, Rubella virus, Borrelia burgdorferi, Multiple sclerosis, MRZ reaction

Introduction

Testing for cerebrospinal fluid (CSF)-restricted total IgG oligoclonal bands (OCB) by isoelectric focusing is a highly sensitive method for detecting intrathecal IgG synthesis, but does not take into account antibody specificity. By contrast, antibody indices (AI) are assessed to test for antigen-specific intrathecal IgG synthesis [1]. It is unclear whether AI assessment is useful in OCB-negative patients. Currently, clinicians often do not test for virus- or bacterium-specific AIs on cost grounds if evidence for intrathecal total IgG synthesis as detected by OCB testing is missing.

Back in 1992, however, Felgenhauer and Reiber reported a positive varicella zoster virus (VZV)-specific AI in 6 total-IgG OCB-negative patients with VZV gangliositis as well as in 4 total-IgG OCB-negative patients with VZV meningitis, suggesting that AI calculation might be more sensitive than total-IgG OCB testing [2]. Here, we report on 21 total-IgG OCB-negative patients with positive AI results for either herpes simplex virus (HSV), VZV, cytomegalovirus (CMV), measles virus (MV), rubella virus (RV), or Borrelia burgdorferi (Bb), thereby providing independent and corroborative evidence for Felgenhauer and Reiber’s hypothesis (see Table 1 for details).

Methods and results

Testing for OCB was performed by a CSF laboratory with long-standing expertise in the field that takes part in the official German external quality assessment organized by INSTAND e.V. twice a year. OCB were determined by isoelectric focusing followed by anti-IgG immunofixation (Sebia, Fulda, Germany). Serum and CSF levels of antibodies to HSV, VZV, CMV, MV, RV, and Bb were assessed using commercially available enzyme linked immunosorbent assays (Enzygnost, Siemes Healthcare, Eschborn, Germany). Total-IgG and total albumin concentrations in CSF and serum were

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Table 1 Patient data and antibody indices to six viral antigens in CSF samples negative for total-IgG oligoclonal bands

| No. | Age | Sex | OCBs | AI | Al | AI | AI | Al | AI | AI | AI | AI | Al | Al | Al | Al | Al | CSF | Suspected diagnosis and indicated by the sender |
|-----|-----|-----|------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|------------------------------------------------|
| #1A | 32  | M   | NEG/NEG | Not det. | 0.72 | 0.74 | Not det. | 0.69 | 0.77 | 1557 | CSF PCR positive HSV encephalitis |
| #1B | 32  | M   | NEG/NEG | Not det. | 43.40 | 1.24 | Not det. | ND | ND | 1456 | CSF PCR positive HSV encephalitis, follow-up 19 days later |
| #1C | 32  | M   | NEG/NEG | ND | 53.40 | 16.30 | ND | ND | ND | 152 | CSF PCR positive HSV encephalitis, follow-up a further 3 days later |
| #2A | 33  | M   | NEG/NEG | Not det. | 2.02 | 1.54 | ND | ND | 0.83 | No data | Not detected for V2, peripheral facial paresis |
| #2B | 33  | M   | NEG/NEG | ND | 1.94 | 1.39 | ND | ND | ND | 19 | Blistering rash V2, peripheral facial paresis, follow-up 8 days later |
| #3  | 30  | M   | NEG/NEG | ND | 2.43 | 1.12 | ND | 0.85 | 1.10 | 2 | No data available |
| #4  | 29  | F   | NEG/NEG | Not det. | 2.12 | 0.99 | 1.30 | ND | 0.94 | 3 | No data available |
| #5A | 34  | F   | NEG/NEG | Not det. | ND | ND | ND | ND | ND | Not data | VZV meningoencephalitis |
| #5B | 34  | F   | NEG/NEG | ND | Not det. | 3.99 | Not det. | ND | ND | 360 | VZV meningoencephalitis, follow-up 5 days later |
| #6  | 60  | F   | NEG/NEG | Not det. | 1.20 | 2.40 | 0.86 | ND | ND | 2 | “FKS06-associated leukoencephalopathy”, grand mal |
| #7  | 51  | M   | NEG/NEG | 1.26 | 1.11 | 2.42 | 1.03 | 0.90 | 1.03 | 1 | “Fatigue and apathy” |
| #8  | 42  | M   | NEG/NEG | Not det. | 1.31 | 3.26 | ND | 1.03 | 1.32 | 3 | “Possible multiple sclerosis” |
| #9  | 74  | F   | NEG/NEG | ND | 1.43 | 2.30 | Not det. | ND | 0.81 | 2 | No data available |
| #10 | 50  | F   | NEG/NEG | ND | Not det. | 2.00 | ND | 0.85 | 0.67 | No data | No data available |
| #11 | 71  | F   | POS/POS* | 20.90 | 1.45 | 1.32 | Not det. | 0.97 | 0.91 | 4 | Neuroborreliosis, encephalitis |
| #12A| 34  | F   | NEG/NEG | Not det. | 2.60 | 0.91 | 0.80 | Not det. | 0.70 | 0.80 | 82 | Neuroborreliosis with abducens and facial nerve paresis |
| #12B| 34  | F   | NEG/NEG | ND | 1.70 | 0.91 | 0.95 | Not det. | 1.15 | 0.83 | 277 | Confirmatory follow-up sample obtained 5 days later |
| #13 | 44  | M   | NEG/NEG | 10.60 | 0.90 | 1.00 | 0.88 | 1.5 | ND | No data | “Aseptic Meningitis” |
| #14 | 84  | M   | NEG/NEG | 5.31 | 1.02 | 0.99 | 0.85 | 1.02 | 1.04 | 2 | No data available |
| #15 | 34  | F   | NEG/NEG | 4.79 | Not det. | 0.99 | Not det. | 0.92 | ND | 1 | No data available |
| #16 | 57  | M   | NEG/NEG | 3.30 | 0.96 | 0.88 | 1.20 | 0.76 | 0.75 | 1 | No data available |
| #17 | 21  | M   | NEG/NEG | 2.50 | Not det. | 0.85 | Not det. | ND | 0.85 | 1 | No data available |
| #18 | 38  | F   | NEG/NEG | ND | 1.67 | 1.00 | 2.40 | 0.44 | 0.94 | No data | “Encephalitis” |
| #19 | 40  | F   | NEG/NEG | Not det. | 16.20 | 1.43 | 0.72 | 0.90 | No data | No data available |
| #20 | 25  | F   | NEG/NEG | Not det. | 0.92 | 1.00 | Not det. | ND | 2.10 | 1 | “Clinically possible multiple sclerosis (Poser)” |
| #21 | 60  | M   | NEG/NEG | ND | 0.85 | 0.87 | 0.87 | 2.80 | 0.92 | No data | No data available |

AI = antibody index; Bb = Borrelia burgdorferi; CMV = cytomegalovirus; HSV = herpes simplex virus; MV = measles virus; NEG/NEG = negative in CSF/negative in serum; not det. = not detectable; ND = not done; OCBs = oligoclonal bands; RV = rubella virus; VZV = varicella zoster virus. * Mirror pattern (so-called “pattern 4” according to an international consensus on OCB diagnostics) in the absence of CSF-restricted IgG bands. † Cross-reactivity between HSV and VZV as a result of herpes simplex blisters in the area innervated by the second branch of the trigeminal nerve were present and the patient developed facial nerve palsy; the result was confirmed in a second CSF/serum samples obtained 8 days after the initial sample was taken.

Additional information:
- Intrathecal synthesis of IgG to HSV, VZV, CMV, MV, RV, and Bb was determined by calculating the respective antibody indices (AI): AI = QIgG[spec]/QIgG[total], if QIgG[total]<QLim, and AI = QIgG[spec]/QIgG[total] if QIgG[total]>QLim, with QIgG[spec]=IgGspec[CSF]/IgGspec[serum], and QIgG[total]=IgGtotal[CSF]/IgGtotal[serum].
- The upper reference range of QIgG[total], was calculated according to Reiber's formula [1].
- No lumbar puncture or phlebotomy was performed for this study, and no stored CSF or serum samples were used for this study. Instead, all data were retrieved retrospectively by an automated database search and analyzed anonymously.
- As clinical data were not available from all patients due to anonymization, a very conservative cut-off for AI positivity (2.0 instead of 1.3) was applied to preclude false-positive results. No other inclusion and exclusion criteria than OCB negativity and presence of a positive AI for any of those six viral and bacterial antigens in the same paired CSF/serum sample were applied. A positive AI was present for Bb in 7 cases (2.0-43.4; median, 4.794) for HSV in 4 cases (2.02-43.4; 2.28), for VZV in 7 (2-16.2; 2.42), for CMV in 1 (2.4), for MV in 1 (2.80), and for RV in 1 (2.10). In case #1, HSV encephalitis was confirmed by PCR and a follow-up lumbar puncture (LP) confirmed the positive AI, again in the absence of total-IgG OCB. In a second case with positive HSV-AI and negative total-IgG OCB, herpes simplex blisters in the area innervated by the second branch of the trigeminal nerve were present and the patient developed facial nerve palsy; the result was confirmed in a second CSF/serum samples obtained 8 days after the initial sample was taken.
later. Confirmation from repeat lumbar puncture was also available in a case of neuroborreliosis (Table 1). All of these cases were associated with CSF pleocytosis. Other clinical diagnoses associated with positive viral AIs as provided by the senders included “encephalitis”, “meningitis”, “abducens and facial nerve paresis”, and “clinically possible multiple sclerosis”; one VZV-AI positive patient was treated with tacrolimus (FK506), a potent immunosuppressant, at the time of LP (Table 1).

To assess the plausibility of the negative total-IgG OCB results, we analysed each patients’ total-IgG CSF/serum ratio (QIgG). Elevated QIgG in the absence of total-IgG OCB positivity would suggest a false-negative OCB result. However, QIgG was found to be normal in all patients (i.e. QIgG < Qlim) as shown in Figure 1.

**Discussion**

These cases confirm that negative total-IgG OCBs in CSF samples might not always predict the absence of positive virus- or bacterium-specific IgG-AIs in patients with suspected CNS infection. Omitting AI testing in OCB negative patients might thus be a possible diagnostic pitfall. In addition to VZV infection [2], positive AIs have previously also been reported in individual total-IgG OCB-negative patients with autoimmune disorders of the central nervous system including anti-GAD antibody positive stiff person syndrome [4], anti-Ri positive paraneoplastic neurological syndromes (PNS) [5], anti-CV2/CRMP5 positive PNS [6], and anti-Yo positive PNS [3].

Around 2-5 % of patients with MS are negative for OCBs. This subgroup, which is small in relative numbers but not so small in absolute numbers due to the high prevalence of MS, is particularly challenging from a diagnostic point of view. A higher sensitivity of antigen-specific AI calculation compared to total-IgG OCB testing might therefore also be of potential relevance for the laboratory diagnosis of multiple sclerosis (MS), since a majority of patients with MS display a polyspecific humoral antibody response to a broad variety of viral and bacterial antigens as determined by AI calculation, with intrathecal antibodies to MV, RV and VZV as its most common constituents (termed MRZR reaction or MRZR), as demonstrated by Reiber and others [7-9].

Apart from MS, a positive MRZR reaction has also been reported in rare cases of lupus and CNS involvement [7]. MRZR testing is currently performed by many CSF laboratories in Germany, but, in our experience, is so far almost exclusively done in OCB-positive patients. In line with the hypothesis that tests for antigen-specific IgG synthesis might be more sensitive than total-IgG OCB detection, Stich et al. recently reported on positive AIs for MV, RV, and VZV in 2/17 (12 %) total-IgG OCB-negative patients with MS but in none of 11 controls. Similarly, the authors found MV-, RV-, and VZV-specific bands in 3/17 (18 %) total-IgG OCB-negative patients with MS as detected by affinity blotting using recombinant viral antigens and a highly sensitive chemiluminescence detection technique [8]. This is in accordance with a report by Frederiksen and Sindic (1998), who found CSF-restricted VZV- and mumps-specific bands in total-IgG OCB-negative patients with MS using a similar type of assay [9].

On the other hand, the fact that a subset of patients with MS presents with a monospecific AI elevation rather than the typical polyspecific, oligoclonal reaction, at least at first presentation [10], and that the spectrum of positive AIs can be broader than just MV, RV, and VZV and include (though less frequently) positive AIs for Bb, toxoplasma and other viral and bacterial agents can pose differential diagnostic challenges in some cases. Accordingly, in patients in whom MS is a reasonable differential diagnosis, AIs for at least the three main constituents of the MRZ reaction (MV, RV, and VZV) should be determined (approximate costs in Germany, ~20-30
In summary, the cases reported here indicate that AI testing should not generally be omitted in total-IgG OCB-negative patients and provides a rationale for systematic and prospective studies on the comparative sensitivity and specificity of AI and total-IgG OCB testing in infectious diseases of the CNS as well as in other indications.

**Abbreviations**

AI: antibody index; Bb: Borrelia burgdorferi; CSF: cerebrospinal fluid; CMV: cytomegalovirus; CNS: central nervous system; HSV: herpes simplex virus; IgG: immunoglobulin G; LP: lumbar puncture; MRZR: measles virus, rubella virus, and varicella zoster virus reaction; MS: multiple sclerosis; MV: measles virus; NB: neuroborreliosis; OCB: oligoclonal IgG bands; PCR: polymerase chain reaction; PNS: paraneoplastic neurological syndromes; Q: ratio; RV: rubella virus; VZV: varicella zoster virus; WCC: white cell count.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

SJ. conceived the study, analysed the data, and wrote the initial draft. P.E. and M.W. conducted and interpreted the laboratory tests; S.J., P.E., B.W., and M.E. revised the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

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