CLINICAL VALUE OF SPECIFIC INTRATHECAL PRODUCTION OF ANTIBODIES

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Summary. – The production of intrathecal antibodies is considered a highly specific marker for an infection of the central nervous system (CNS), e.g. borreliosis or tick-borne encephalitis (TBE). To investigate the validity of this assumption, we examined records of patients who had been hospitalized between 1989 and 1995, who were tested for borreliosis (n = 8003) and TBE (n = 904) and whose cerebrospinal fluid (CSF) had subsequently tested positive for intrathecal production of antibodies. The time period between the beginning of the symptoms and the time of the CSF examination ranged from one day to six weeks. Seventy-seven patients showed a production of intrathecal antibodies against *Borrelia burgdorferi*. Three of these patients were false positives with no history and no clinical signs of neuroborreliosis. In two cases, this was due to a non-specific cross-reaction caused by a preceding infection with syphilis. The third false positive was possibly caused by an earlier administration of immunoglobulins. Three patients showed a production of intrathecal antibodies against TBE virus. Two of these patients were false positives. In one case, we suspect that the production of intrathecal antibodies was caused by a non-specific immune reaction during an acute neuroborreliosis. One year earlier, the patient had contact with TBE virus through a vaccination against TBE. The cause of the second false positive is unclear, the clinical findings, acute encephalitis and the serological analysis suggest a cross-reaction with a virus similar to TBE. A specific intrathecal production of antibodies is not a proof for an infection of the CNS. In unclear cases, one should carry out a Western blot analysis or, if one suspects a case of TBE, a neutralization test.

Key words: neuroborreliosis; tick-borne encephalitis; intrathecal antibodies

Introduction

Lyme-borreliosis is the most common vector-borne infection in Germany. A large epidemiological study carried out in Sweden, a country assumed to have infection rates comparable to Germany, showed an incidence of 69 per 100,000 inhabitants (Berglund *et al.*, 1995). Erythema chronicum migrans was the most frequent clinical manifestation (77%), followed by neuroborreliosis (16%), which continues to pose problems, since its serologic diagnosis is difficult. The cultivation of the bacteria from CSF of patients suspected to suffer from neuroborreliosis is the most reliable way to confirm the diagnosis. Unfortunately, this approach is rarely successful. Proof of IgG antibodies in the serum is not very specific, because a large part of the population has had contact with the bacteria and therefore tests positive for antibodies. During recent years, the enzyme-linked immunosorbent assay of IgM (IgM-ELISA), which was considered to be of low specificity in the past, has become more reliable for routine diagnostic testing (Braune, 1991). For this reason, diagnostics in the past has relied on the amount and increase of antibody titers in CSF and in serum in particular. However, due to insufficient standardization of testing procedures, the measured titers show a large inter-laboratory variability. New techniques such as the polymerase chain reaction (PCR) and Western blot analysis have improved their reliability (Karch *et al.*, 1994; Luft *et al.*, 1992).

Abbreviations: CNS = central nervous system; CSF = cerebrospinal fluid; ELISA = enzyme-linked immunosorbent assay; IAI = intrathecal antibody index; PCR = polymerase chain reaction; TBE = tick-borne encephalitis; TPHA = *Treponema pallidum* haemagglutination

So far, the most reliable diagnostic tool to detect an infection involving the CNS is the intrathecal antibody index (IAI) test (Halperin *et al.*, 1991; Hammers-Berggren *et al.*, 1993; Hansen and Lebech, 1992; Zbinden *et al.*, 1993). Nevertheless, the sensitivity of this test has been questioned, because false negatives, such as a clinically manifest borreliosis with a negative IAI test, have been reported (Cooke, 1992; Oksi *et al.*, 1993; Pachner *et al.*, 1989). False positives have also been observed in the use of PCR, which gave positive results when testing the skin, while ELISA, Western blot analysis and clinical history ruled out borreliosis (Schempp *et al.*, 1993). On the other hand, the specificity of the IAI test for an infection of the CNS has not been questioned and a proof of the production of intrathecal antibodies was considered an almost certain proof of neuroborreliosis (Braune, 1991; Halperin *et al.*, 1991; Wilske *et al.*, 1991). Specificity rates reported range from 98% to 100%, depending on the investigator (Mauch *et al.*, 1990; Wilske *et al.*, 1991).

In the present study, we analyzed borreliosis and TBE tests carried out in our clinic between 1989 and 1995 and investigated the causes of false positive IAI results.

Materials and Methods

In the present study, 8003 sera of patients admitted between 1989 and 1995 to our neurological clinic were tested serologically for antibodies against borrelia. The tests used were a *Borrelia burgdorferi* ELISA and a Western blot analysis (both from Genzyme Virotech, Rüsselsheim, Germany). During the same time period, 904 patients were tested serologically for TBE using a TBE IgG-ELISA and a Western blot analysis (both from Immuno, Heidelberg, Germany).

Sera and CSF were kept frozen at -20°C. The sera and CSF tests were normally carried out on the day of admission to the hospital. The time period between the beginning of the symptoms and admission to the hospital ranged from one day to six weeks. In 75% of the cases, a second lumbar puncture was carried out ten days after the first one.

IAI, a serum/CSF quotient which is an indicator of intrathecal antibody production, was computed from the results of IgG-ELISA (Heller *et al.*, 1990; Holzer *et al.*, 1988). This index served as a quantitative measure of intrathecal synthesis of antibodies against the virus and was computed as follows:

$$IAI = \frac{\text{CSF titer}}{\text{Serum titer}} : \frac{\text{CSF IgG}}{\text{Serum IgG}}$$

An index larger than 2.5 was considered positive.

Results

Borreliosis

Of 8003 sera tested for borrelia antibodies, 1833 (22.9%) were positive in IgG-ELISA, 1509 (18.9%) were borderline and 4661 (58.2%) were negative. In addition, 1854 samples of CSF were tested, 430 of which tested positive for IgG antibodies. For 77 patients, a positive IAI was computed. Of these 77 patients, three showed no clinical signs or a history of neuroborreliosis:

Case 1: A 56-year-old male patient underwent stationary treatment for two months for a psycho syndrome. His CSF cell count was 6/3 per µl, protein concentration was 68 mg/dl. The Reiberpattern showed a border deviation (Reiber, 1980). The isoelectric focussation showed identical bands in serum and CSF. The borrelia IgG titer was positive in serum and CSF (1:230 vs. 1:4). The IAI was 3.1, and the IgG and IgM Western blots were negative. Syphilis diagnostics in serum and CSF yielded reactive TPHA (*Treponema pallidum* haemagglutination) and IgG FTA-ABS (fluorescent treponemal antibody-absorption) tests. The VDRL (venereal disease research laboratory) test was negative, and the ELISA showed syphilis IgM antibodies in the serum.

Case 2: A 64-year-old male patient underwent stationary treatment for an infarction in the left brain. Because the inflammatory parameters were elevated, the suspected cause of the stroke was vasculitis. The CSF cell count was 10/3 per µl, protein concentration was 47 mg/dl. The Reiber pattern showed a border deviation. The isoelectric focussation was positive and showed identical bands in CSF and serum. The borrelia IgG titer was positive in serum and CSF (1:280 vs. 1:2.9). The IAI was 2.8, and the IgG and IgM Western blots were negative. Syphilis diagnostics showed reactive TPHA and IgG FTA-ABS tests, but a negative VDRL test.

Case 3: A 32-year-old male patient underwent stationary treatment for a 4-day-old paresis and problems with swallowing. Two weeks earlier, he had suffered from a gastro-intestinal infection with fever. The CSF cell count was 32/3 per µl, protein concentration was 46 mg/dl. The Reiber pattern and the isoelectric focussation were negative. Because of the clinical symptoms, a polyradiculitis was diagnosed. After a daily treatment with 40 g of immunoglobulins (Venimmun®) for six days, the paresis improved slowly. An additional spinal tap one week later showed 20/3 cells per µl and a protein concentration of 59 mg/dl. On admission, borrelia serology was negative in serum and CSF. After one week, IgG antibodies (1:360 vs. 1:2) were detected in serum and CSF, the IAI test was positive at 2.8, and the IgG and IgM Western blots were negative.

TBE

Of 904 serum samples, 47 (5.2%) tested positive and 134 (13.8%) borderline for TBE IgG. One patient, whose serum tested positive for both IgG and IgM, also tested positive for IgG and IgM in CSF. Another CSF sample was positive, 7 were borderline in the IgG-ELISA.

Three of the patients tested for TBE virus antibodies showed a positive IAI test, one patient who tested positive for IgG and

IgM suffered from a clinically manifest TBE, which was acquired through a tick bite in the Black Forest, an endemic region in Southern Germany. The two other patients with a positive IAI test showed no symptoms and no history of TBE:

Case 4: A 35-year-old female patient was admitted with paresis of the facial nerve. The CSF cell count was 1136/3 per µl, protein concentration was 94 mg/dl. The isoelectric focussation showed oligoclonal bands. Serum and CSF showed TBE IgG antibodies in ELISA and Western blot analysis (Immuno GmbH, Heidelberg). The IAI was 5.9. The IgM-ELISA was negative in serum and CSF. The borrelia IgG serum antibody titer was borderline (1:150), the CSF titer positive (1:25). The borrelia IAI was 44. During a 21-day treatment with Ceftriaxone (Rocephin®), the paresis of the facial nerve disappeared. The patient had been vaccinated against TBE in 1991 and had repeatedly suffered tick bites.

Case 5: A 58-year-old man was admitted with fever and problems with orientation and coordination, which had begun suddenly. During his six-week stay in the hospital, he underwent seven spinal taps. The cell count (dominated by lymphocytes) ranged from 116/3 to 488/3 per µl, protein concentration ranged from 56 to 212 mg/dl. A MRI showed dense areas with an affinity for gadolinium in both temporal lobes. The serum TBE IgG-ELISA was negative in the beginning and turned positive later. The IAI was negative on admission and rose continuously within two weeks to 10.3 and declined subsequently to 7.4. The TBE IgG Western blot was negative. The TBE IgM-ELISA was negative for serum and CSF. The herpes simplex IgG antibody titer remained constant at 320. The borrelia IgG titer was positive in serum, the IAI was negative. The patient had never been vaccinated against TBE, did not remember a tick bite and had not left his home region (Saarland), in the preceding three months. An indirect immunofluorescence test for possibly cross-reacting flaviviruses (yellow fever, dengue, West Nile) was negative.

Discussion

In the past, a positive IAI and an intrathecal production of antibodies was considered a highly specific marker for an infection of CNS. In our study, we examined 5 cases of false positives, in which patients had no clinical manifestation of a CNS infection, and where no causative agent could be determined serologically.

In cases 1 and 2, the false positive results were due to a cross-reaction with the syphilis bacterium, a source of error which has been known for some time (Wilske *et al.*, 1991). For this reason, a syphilis infection should be ruled out before neuroborreliosis is diagnosed from the serological results alone. In case 2, an additional vasculitis may have lead to a non-specific production of IgG.

In case 3, we cannot exclude with certainty that the patient suffered from neuroborreliosis. However, taking into account the preceding gastro-intestinal infection, the clinical symptoms, the rapid improvement, and the short timespan between the clinical examinations and the results of the Western blot analysis, we suspect a connection between the positive IAI and the administration of immunoglobulins, although we have no convincing pathophysiological explanation for this phenomenon.

Case 4 was a neuroborreliosis which, surprisingly, showed a highly positive IAI for both borreliosis and TBE. A vaccination against TBE four years earlier could explain the TBE-positive IgG-ELISA and Western blot analysis. Yet, according to the literature reviewed by us, an intrathecal TBE IgG antibody synthesis has only been observed in patients suffering from TBE, but not in vaccinated patients. Possibly, a CNS infection could lead to a non-specific response of the immune system with an increased production of other antibodies that were not related to the current infection, as long as the CNS has had contact with these agents in the past.

Case 5: The fact that the intrathecal synthesis of IgG antibodies was demonstrated in the ELISA, but not in the Western blot analysis is difficult to interpret. Clinical and CSF findings suggest a viral meningoencephalitis. A herpes encephalitis, first diagnosed on the basis of the MRI results, is an unlikely cause, because of the constant herpes simplex IgG titer. Neuroborreliosis is another possible diagnosis, considering the positive borrelia titer, but a cerebral manifestation of borreliosis is unlikely, because of the negative IAI. For this reason, we suspect that a virus similar to TBE, which cross-reacted in the ELISA, lead to a viral meningoencephalitis. We were unable to determine the causative agent.

In general, the production of specific intrathecal antibodies is a reliable indicator of a CNS infection. However, cross-reactions should be ruled out. Case 4 shows that in individual cases, a non-specific immune reaction or re-activation can occur. If the IAI is positive for several potential causative agents, the highest value indicates the one most likely to be the cause of infection.

When investigating the IAI, one has to consider that the demonstration of a positive IAI is only possible during a certain time interval between infection or the beginning of the clinical symptoms and the analysis of the serum/CSF sample.

Hansen and Lebech (1991) examined 100 patients suffering from neuroborreliosis. Of the patients examined in the first week after the onset of neurological symptons, 17% showed a specific production of intrathecal IgG antibodies. In the second week, 82% of the patients showed a specific antibody production. By the the sixth week, 100% of the patients produced specific antibodies. This shows that the specific intrathecal antibody response normally begins in the second week after the onset of neurological symptoms.

Hammers-Berggren *et al.* (1993) were able to show in 26 of 27 patients with neuroborreliosis a specific production of intrathecal antibodies. The time interval between the beginning of the neurological symptoms and the examination of the CSF samples ranged from 2 weeks to 2 years.

In a large number of patients examined by us in this study, the time interval between the occurrence of the symptoms and the lumbar puncture was shorter. Therefore, a part of the patients would probably have shown a positive IAI, if an additional lumbar puncture had been carried out at a later time point.

To clarify the diagnosis as quickly as possible, a lumbar puncture was usually carried out as soon as possible, usually on the first day of admission, and patients with clinical signs of a neuroborreliosis and characteristic CSF status were subsequently treated on the first day with antibiotics. For this reason, it is possible that some of the patients who were treated early never developed a positive IAI. Because the strategy of the therapy is normally a rapid treatment, there are no studies concerning this question. Since our study is a retrospective investigation, it was not possible to always chose the optimal time interval.

The specificity of tests for intrathecal production of antibodies during a CNS infection depends decisively on the tested group and the incidence of the disease. Among 77 borrelia tests, we discovered 3 false positives, whereas 2 out of 3 TBE tests were false positives. This can be explained by the fact that the incidence of neuroborreliosis in our region (Saarland) is much higher than the incidence of TBE (Treib, 1994; Treib *et al.*, 1996). In unclear cases, one should carry out a Western blot analysis or, when the suspected diagnosis is TBE, a neutralization test.

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