CASE REPORT

Seronegative lyme neuroborreliosis in a patient using rituximab

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SUMMARY

A 66-year-old woman presented with severe shooting pains throughout her back and legs, followed by progressive deafness, weight loss and headache. She had a history of marginal zone B-cell lymphoma stage IV-B, for which she was successfully treated with immunochemotherapy and rituximab maintenance therapy. A relapse was suspected, but chemotherapy was not administered, since, despite elaborate investigations, malignancy could not be proven. Because of a history of tick bites she was tested for antibodies against *Borrelia burgdorferi* in serum and cerebrospinal fluid (CSF), which were negative. However, a *B burgdorferi* PCR on CSF came back positive. The patient was treated for seronegative Lyme neuroborreliosis with ceftriaxone intravenously and dramatically improved. This case presentation demonstrates that, in immunocompromised patients, it is important not to solely rely on antibody testing and to use additional diagnostic tests to avoid missing or delaying the diagnosis.

BACKGROUND

Lyme borreliosis is a spirochetal infection caused by *Borrelia burgdorferi* sensu lato and transmitted by ticks. The clinical manifestations of Lyme borreliosis can be divided into three phases: early Lyme borreliosis and early and late disseminated Lyme borreliosis. Early Lyme borreliosis is characterised by erythema migrans, which is an expanding erythematous skin lesion with central clearing located at the site of the tick bite, typically emerging after 7–14 days.¹ When the infection is left untreated, the spirochaete can disseminate and cause Lyme neuroborreliosis or various cutaneous and musculoskeletal manifestations.^{2–3} In Europe the most common manifestation of early Lyme neuroborreliosis is a painful meningoradiculitis, sometimes accompanied by cranial neuropathy, also called Bannwarth's syndrome. This usually occurs few weeks to several months after the tick bite and may be the first manifestation of Lyme borreliosis.

For the diagnosis of Lyme neuroborreliosis analysis of serum and cerebrospinal fluid (CSF) is required. European Lyme neuroborreliosis is associated with pleiocytosis, typically with 10–1000 white blood cells/mm³.⁴ A normal cell count in the CSF is rare, but can be present in the very early stages in immunosuppressed individuals, or occasionally in patients with long-lasting symptoms.⁵ The diagnosis of Lyme neuroborreliosis is confirmed by the demonstration of intrathecal

production of anti-*Borrelia* antibodies as shown by an increased CSF/serum antibody index. Early in the course of neuroborreliosis the sensitivity of this antibody index is 55–80%.^{5–6} After 6 weeks of symptoms the sensitivity of the antibody index approximates 100%.^{5–9} The diagnostic sensitivity of a (PCR) on CSF is low (10–50%), the specificity however is high (98–100%), provided precautions are taken to avoid contamination.^{8–10} Furthermore, it is possible to culture the spirochaete from CSF, but owing to low sensitivity, slow growth of the spirochaetes—cultures should be monitored for up to 8–12 weeks—and restriction to few specialised laboratories, this method is rarely used.

Antibiotics are effective for all manifestations of the disease and the prognosis is usually excellent,¹¹ although in a minority of patients debilitating residual aspecific complaints may persist for several months. Little is known about the presentation, outcome and treatment efficacy of *Borrelia* infection in immunocompromised patients, although some studies suggest these are comparable to non-immunocompromised patients.^{12–13} In the case presentation described in this report it is demonstrated that, when B-cells are depleted by rituximab, serology for Lyme borreliosis can remain negative even 6 weeks after the onset of symptoms. Therefore, in immunocompromised patients, it is important not to solely rely on antibody testing and to use additional diagnostic tests to avoid missing or delaying the accurate diagnosis.

CASE PRESENTATION

A 66-year-old woman presented with acute onset of neurological symptoms. Her medical history revealed diverticulitis, sterilisation and an appendectomy. Besides, in the past she had been bitten by ticks several times during her outdoor activities, such as hiking and camping in the Netherlands. The last tick bite she remembered was more than a year before her first visit to the haematologist. She had always promptly removed the ticks herself and she had never noticed any redness later. In April 2009, she was diagnosed with an extranodal marginal zone B-cell lymphoma stage IV-B, with extensive localisations in the abdomen, bladder wall, left conjunctiva, bone marrow, stomach and pleurae. At that time, a biopsy was taken from an erythematous skin lesion on her leg (figure 1) to exclude lymphoma localisation and to test for the presence of *Borrelia* spirochaetes by immunostaining. Histopathology showed a perivascular infiltrate mainly consisting of lymphocytes and a negative

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Figure 1 Erythematous skin lesion on the right leg (April 2009).

immunostaining for *Borrelia*. Also, the *B burgdorferi* C6-enzyme immune assay (C6-EIA) performed on serum turned out to be negative. Finally, a skin culture for *Borrelia* in modified Barbour–Stoenner–Kelly medium remained negative. There was no evidence of lymphoma localisation in the skin lesion either. The patient was treated with three 21-day cycles of immunochemotherapy consisting of rituximab, cyclophosphamide, vincristin and prednisone. At restaging after three cycles the disease was stable. She continued with six cycles of R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristin and

Prednison), of which the last one was administered in November 2009. Because of a good partial response, maintenance treatment with rituximab 375 mg/m² once every 3 months was initiated. A total body positron emission tomography-CT scan (PET-CT) in April 2010 did not show active disease.

In September 2010, she presented at the emergency department with sudden onset of severe shooting pains in her back and both legs, followed by progressive deafness, nausea, vertigo, a headache and 5 kg loss of weight. Physical examination revealed signs of dehydration and a temperature of 37.7 °C. There were no signs of paresis, disturbed coordination or ataxia. Tendon reflexes were low or absent. Babinski reflexes were positive in both feet and there was only minor preexistent (putatively vincristine-induced) hypesthesia of the fingertips and soles of her feet. There were no signs of arthritis, lymphadenopathy or skin lesions. She was admitted to the department of internal medicine for further analysis.

INVESTIGATIONS

Table 1 shows relevant laboratory findings in peripheral blood at admission (table 1). X-rays of the pelvis and legs did not demonstrate osteolytic lesions. Additional blood tests revealed no IgM/IgG-antibodies against *B burgdorferi* and a PET-CT-scan showed no signs of tumour activity or compression of the myelum. An MRI of the brain and spinal cord showed no evidence of leptomeningeal metastases. A bone marrow biopsy showed a normocellular bone marrow with normal differentiation of all cell lines, and no evidence of lymphoma. Two lumbar punctures were performed. Marked pleiocytosis was observed in the first CSF sample (table 1 and figure 2A) with atypical lymphoid cells with an extensive amount of cytoplasm and enlarged irregular eccentric nuclei, morphologically suggestive of malignant degeneration (figure 2B). Immunohistochemical stainings unfortunately were indeterminate, and clonality could not be demonstrated by flow cytometry. An occult leptomeningeal localisation of the lymphoma was considered. However, because the imaging studies did not show any signs of systemic relapse, and the cytological findings could not be confirmed by demonstration of clonality, chemotherapy was not administered and an infectious cause was considered. Thus, CSF was cultured for bacteria, but cultures remained negative and PCRs for a wide range of neurotropic viruses also proved to be negative (table 1). In addition, no anti-*B burgdorferi* IgM/IgG antibodies were demonstrated in the CSF. After a

Table 1 Relevant laboratory results at the time of admission, 3 weeks after symptoms appeared

Serum		Cerebrospinal fluid (CSF)		Virology on CSF	
Hb (mmol/l)	7.1	Aspect	Clear	Cytomegalovirus PCR	Negative
MCV (fl)	93.6	Erythrocytes (cells/3 µl)	<900	Epstein-Barr virus PCR	Negative
WBC (10 ⁹ /l)	5.1	WBC (cells/3 µl)	492	Herpes-Simplex virus PCR	Negative
Platelets (10 ⁹ /l)	321	PMN (%)	11	Varicella Zoster virus PCR	Negative
Neutrophils (10 ⁹ /l)	3.53	MN (%)	89	Enterovirus PCR	Negative
Lymphocytes (10 ⁹ /l)	0.34	Protein (g/l)	1.42	Parechovirus PCR	Negative
Monocytes (10 ⁹ /l)	0.42	Glucose	1.7	JC virus PCR	Negative
Eosinophils (10 ⁹ /l)	0.05				
Basophils (10 ⁹ /l)	0.01				
Na ⁺ (mmol/l)	132				
K ⁺ (mmol/l)	3.7				
Creatine (µmol/l)	58				
ESR (mm/h)	9

Figure 2 (A) Pleiocytosis with many atypical lymphoid cells. (B) The enlargement demonstrates cells with an extensive amount of cytoplasm and enlarged irregular eccentric nuclei (arrows), suspicious for malignant degeneration.

first round of negative tests, the CSF was also tested for DNA of *B burgdorferi* by PCR, which resulted positive. This result was confirmed by a PCR on a CSF-sample taken 2 weeks later (in an independent laboratory). A *Borrelia* culture of CSF was performed that was found to be negative.

TREATMENT

With a diagnosis of seronegative Lyme neuroborreliosis, our patient was treated with ceftriaxone once daily 2000 mg intravenously for 3 weeks.

OUTCOME AND FOLLOW-UP

During these 3 weeks she improved dramatically, which further strengthened the diagnosis. She gained weight, the complaints of headache and vertigo disappeared and her hearing loss slowly improved. Paired serology on blood and CSF 7 weeks later still revealed no antibodies against *B burgdorferi*.

One-and-a-half years later, a routine MRI of the myelum showed no evidence for relapse of the lymphoma and the hearing ability had completely returned. During neurological follow-up the patient reported a slight unsteadiness of gait, which was attributed to residual postinfectious complications.

The rituximab maintenance therapy had been discontinued during her hospital admission, but despite the slow repopulation of B-lymphocyte subsets, 1.5 years after her initial presentation, there were still no antibodies against *B burgdorferi* (figure 3).

DISCUSSION

This patient reported a history of tick bites and suffered weight loss and fatigue, and more specifically from a cranial neuropathy (hearing loss) and meningoradiculitis (headache and shooting pains), which were highly suggestive of Lyme neuroborreliosis. However, at that time, because of her history of B-cell lymphoma, 5 kg of weight loss and an elevated ESR, relapse of her lymphoma with leptomeningeal localisation was considered to be the most likely cause of her neurological complaints. This hypothesis was strengthened by absence of anti-*Borrelia* antibodies in both serum and CSF, and the presence of highly atypical lymphocytes in CSF, suggestive of malignancy. However, imaging of the brain and myelum did not show evidence of leptomeningeal lymphoma localisation, and clonality of the atypical lymphocytes in the CSF could not be proven. In contrast, *B burgdorferi* PCR on CSF, which is known to have a sensitivity of only 10–50%, demonstrated the presence of *B burgdorferi*

Figure 3 A (non-linear) timeline demonstrates the time between clinical events (top) and results of laboratory tests (bottom). The dates for different laboratory tests represent the dates samples were obtained. The results were obtained several days (PCR) or weeks (culture) later. *This cerebrospinal fluid sample was tested for *Borrelia* PCR on the 13 October 2010, after PCRs for a wide range of neurotropic viruses all proved to be negative.

DNA in CSF, which was confirmed in a consecutive CSF sample 2 weeks later. This, combined with the fact that the patient's complaints disappeared upon treatment with ceftriaxone, strongly supports the diagnosis of Lyme neuroborreliosis.

The sensitivity of serology approximates 100% for (disseminated) Lyme borreliosis with a duration of longer than 6–8 weeks. To our knowledge only one case report has described seronegative Lyme neuroborreliosis in a patient who was treated with rituximab (for chronic lymphatic leukaemia).¹⁴ That particular patient had a typical erythema migrans 1 week before the onset of neuropathic pain, and analysis of the CSF showed pleiocytosis. Although no anti-*Borrelia* antibodies could be detected in the serum of this patient, retrospective PCR analysis of the CSF sample confirmed the clinical diagnosis. However, serology can be negative when symptoms are of short duration (<6–8 weeks) also in immunocompetent individuals. That case does not report *Borrelia* serology at later time-points. Negative tests for leishmaniasis antibodies during treatment with rituximab have also been described in a patient who had been treated for non-Hodgkin's lymphoma and was on maintenance therapy with rituximab. This patient died of undiagnosed visceral leishmaniasis.¹⁵

In retrospect, this patient might have presented with an atypical erythema migrans at the time she was diagnosed with extranodal marginal zone B-cell lymphoma stage IV-B, for which she was started on chemotherapy in combination with the anti-CD20 monoclonal antibody rituximab. At that time erythema migrans was considered unlikely because of negative serology, a negative skin biopsy *Borrelia* culture and a negative *B burgdorferi* staining of a skin biopsy section. In retrospect, a PCR on deparaffinised skin sections did not reveal *B burgdorferi* DNA. The sensitivity of *B burgdorferi* serology in erythema migrans varies from 38 to 88% depending upon the duration of the erythema, which may explain why the patient did not have detectable anti-*Borrelia* antibodies at that time. Rituximab is known to deplete normal B-lymphocytes and this is likely to have hampered further production of antibodies against *B burgdorferi* not only in serum, but also in CSF.

In conclusion, this case report is an important reminder of the fact that in patients treated with drugs such as rituximab, which interfere with the immune response, serological diagnostics are not always reliable. In immunocompromised patients with a high suspicion of infectious diseases, such as infection with *B burgdorferi*, it is therefore important not to rely solely on antibody testing but to use several additional diagnostic tests (e.g. PCR and culture) to avoid missing or delaying the diagnosis. Lyme neuroborreliosis, although sometimes easy to diagnose, can be difficult to diagnose, and when the clinical suspicion is high, like in our case, additional diagnostic tests or sometimes even empiric treatment should be considered.

Learning points

- Depletion of B-cells by rituximab can lead to false negative serological test results.
- In patients using rituximab, additional diagnostic tests are sometimes needed to confirm a diagnosis.
- When suspicion for Lyme neuroborreliosis is high, but the diagnosis cannot be confirmed by routine diagnostic tests, and other causes have been excluded, empiric treatment should be considered, especially in immunocompromised patients.

Competing interests None.

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