Author's response to reviews

Title: Unilateral phrenic nerve lesion in Lyme neuroborreliosis

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Author's response to reviews: see over
Dear Editorial team,

Thank you very much for your e-mail dated November 14th, 2012. We are happy to see that both reviewers provided basically positive comments and did appreciate the importance of our study. We carefully modified the manuscript in accordance with their suggestions. Please find enclosed a point-by-point response to the reviewers’ comments. In the revised manuscript all changes are highlighted. We hope that this revised version of our manuscript will be suitable for publication in BMC Pulmonary Medicine and look forward to hearing from you.

Sincerely yours

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Reviewer 1:

(i) The antibody index is IgG positive (ratio >1.5), but not the IgM (ratio 0.8).
(ii) IgM antibodies were not detected in the CSF.

Acute Lyme neuroborreliosis (LNB) is a clinical diagnosis supported by characteristic CSF abnormalities, in particular elevated CSF leukocyte counts, blood-CSF barrier dysfunction, predominant intrathecal IgM synthesis and an elevated Borrelia burgdorferi (BB)-specific antibody index (AI) for IgM or IgG (Djukic et al. 2012, Rauer 2012).

To diagnose definite acute LNB in Germany, according to the criteria of the German Society of Neurology (DGN) and neurological societies of other European countries, the detection of intrathecal synthesis of specific BB antibodies [either IgG or IgM or both (IgG and IgM)] is necessary (Hansen et al. 1992, Rauer 2012, Blanc et al. 2007). The detection of specific IgG anti-BB antibodies in CSF alone also indicates acute LNB (Djukic et al. 2012).

The immune response within the CNS during acute LNB typically has a strong B-cell component resembling neurosyphilis (Halperin 2011). This in turn results in intrathecal production of anti–BB antibody, i.e. local production of specific antibody in excess of those which enter the CSF from the blood (Halperin 2011). Finally, this intrathecal production of antibodies can result in an elevated total IgM or IgG concentration in the CSF (increased IgM or IgG index or synthesis rate) and oligoclonal bands (Wang et al. 1993).

The presence of an intrathecal production of BB-specific antibodies in our patient was evaluated by the calculation of the BB-specific antibody index (AI). For this purpose, a modified immunoassay was used, based on the commercially available immunoassay plates Enzygnost Borreliosis IgG/IgM of Siemens Healthcare Diagnostics GmbH, Marburg, Germany. To increase the sensitivity of the assay the secondary antibodies supplied with the test kit were replaced by anti-IgG or anti-IgM antibodies from DAKO, Hamburg, Germany. As previous studies showed, the BB-specific intrathecal IgG production in CSF appears to be more frequent than the BB-specific intrathecal IgM production when enzyme
immunoassays are used (Djukic et al. 2012, Wang et al. 1993, Hansen et al. 1991). One reason is that the antibodies (in particular IgG and IgM) compete with the binding site of the antigens on the enzyme-linked immunosorbent assay (ELISA) plates. The IgG molecules are smaller than the IgM molecules and have better potential to bind to the antigens. IgG can displace the IgM from the binding sites of the antigens, which result in an underestimation of the BB-specific intrathecal IgM production (Reiber 2005).

(iii) BB PCR was negative
The negative CSF polymerase chain reaction (PCR) test for BB-DNA does not exclude the diagnosis of acute LNB because the sensitivity of PCR in CSF is very low (Aguero-Rosenfeld et al. 2005, Lebech et al. 2000). To establish the diagnosis in patients with LNB, the measurement of the specific intrathecal antibody synthesis is superior to PCR (Lebech et al. 2000, Rauer et al. 2012).

(iv) Was CSF CXCL13 examined?
No.
In recent years, the B lymphocyte chemoattractant chemokine CXCL13 has been identified as a potentially important biomarker for the diagnosis of acute LNB (Schmidt et al. 2011, Ruprecht et al. 2005). Reported sensitivities and specificities are high [in a similar range as for the BB-specific antibody index (AI)] suggesting that CSF CXCL13 might be a useful addition to the diagnostic armamentarium, especially in the very early stage of LNB, where AIs might still be negative. However, CXCL13 seems to be the major determinant for B cell recruitment to the CNS compartment in different neuroinflammatory diseases and not only in LNB (Kowarik et al. 2012). CXCL13 levels in the CSF rather reflect a strong humoral immune response in the CNS compartment than being specific for a particular disease entity (Kowarik et al. 2012). As patient numbers in the published studies are rather low, further corroboration of these results is needed.

(v) Despite 2 weeks of iv cefalosporin treatment which resulted in clinical improvement in other neurological symptoms, the diaphragmatic weakness
persisted, even after a further course of oral doxycycline. This all suggests that the LNB may not be entirely responsible for the diaphragmatic dysfunction.

Treatment with antimicrobial regimens is usually effective in Lyme disease (Halperin et al. 2011) but some symptoms may persist longer despite antibiotic treatment and the recovery is incomplete (Mygland et al. 2006, Ackermann et al. 1988). One year after successful treatment of LNB residual facial paresis persist in about 5% (Kaiser 2004).

The serology suggests exposure to Lyme but does not in my opinion confirm acute infection. The authors should address these concerns.

This was done.

2. The authors explanation axonal damage is responsible for the delayed phrenic nerve recovery is speculative and not convincing. Lyme-induced neuropathy is considered to be a direct perineuritic infiltration, and response to antibiotics is typically brisk.

The sentence concerning axonal injury was eliminated.


Neurophysiologic studies in patients with LNB indicate that all these patients have a mononeuropathy multiplex—ranging from facial nerve palsy to what is known as a confluent mononeuropathy multiplex—the sort of multifocal process thought to underlie many diabetic neuropathies (Halperin 2011). This is further supported by observations in the rhesus macaque monkey model, in which virtually all experimentally infected monkeys develop neurophysiologic and pathologic evidence of a mononeuropathy multiplex (England et al. 1997).
References


**Reviewer 2:**

1) In the case presentation was the shooting thoracic pain left sided?

   Yes. This was modified.

2) Were the dysarthria and dysphagia thought due to the facial palsy or other cranial nerve involvement.

   The dysarthria and dysphagia were due to the facial palsy. Other cranial nerves (except the N. abducens) were not involved.

3) Was the MRI done without and with contrast?

   Yes, the MRI was done without and with contrast. This was added.

4) This is a question of personal interest as I cannot imagine that the CSF signal on FLAIR imaging was normal with a CSF protein of 1324mg/l were the images reviewed by a neuroradiologist?

   Yes, the images were reviewed by a neuroradiologist (University Medical Centre Goettingen, Department of Neuroradiology). The FLAIR imaging was unremarkable.

   The MRI findings in LNB are usually focal lesions in the white matter of the brain (Halperin et al. 1989; Agarwal and Sze 2009). The nerve-root or meningeal enhancement can be
detected but is rare (in a recent study three of 66 patients demonstrated nerve-root or meningeal enhancement) (Agarwal and Sze 2009).

5) I would write 129 cells/µl

   This was changed.

6) The CT scan of the chest was done to exclude a mass lesion not show the elevation of the hemidiaphragm. The language is wrong.

   This was corrected.

7) "In this case" I might say something to the effect that the diaphragmatic paralysis became evident after the presentation with more typical acute Lyme meningitis and cranial nerve palsies.

   This was modified.

To my mind he was adequately before the presentation with dyspnea. Was a chest X-ray done on his first admission?

   A chest X-ray was not done on his first admission.

8) It is not necessary to say that the diaphragmatic paralysis is a serious condition.

   The word “serious” was deleted.

9) Diaphragmatic weakness has been associated with trauma, malignant compression/infiltration, metabolic, inflammatory and other disorders, but rarely with Lyme disease.

   This sentence was added.

10) The CSF indicates meningitis one doesn’t do an LP to exclude disc prolapse and this should be modified.

    This was modified accordingly.

11) LNB should be considered in the differential diagnosis of respiratory failure (due to diaphragmatic paralysis)

    This was modified.

12) There are too few cases to comment on outcome, but ability to treat is the issue.

    This was modified as suggested.
13) The case should be reported as the more frequently it is reported the more likely it is to be recognized and treated when it presents as an isolated syndrome not in the context of acute Lyme meningitis. This diagnosis has not made it into the lists of a host of textbooks I checked as I was writing my comments.

We added to the conclusion:

“It is important in clinical practice to consider Lyme neuroborreliosis also in patients presenting with respiratory failure as an isolated syndrome.“

References
