

## **Diagnostics of Neuroborreliosis (NB) from the Real World in Real Numbers – Time to Abandon the EFNS Guidelines!**

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### **Introduction.**

NB is the main manifestation of disseminated borreliosis with protean clinical presentation. The diagnosis depends on clinical findings supported by laboratory investigations. The case definition mostly used in Europe, has been proposed by EFNS depend heavily on clinical findings and serology. The suggested diagnostic process generates a large group of possible cases of NB, resulting in a considerable number of unsure patients, who are exposed to treatments of questionable relevance or getting no treatment at all. The poor performance of objective diagnostic tests is a major drawback not without consequences. In the Åland Islands the public awareness of tick-borne diseases is very high and the annual infection incidence is  $>1500/10^5$  inhabitants. This epidemiological situation results in a seroprevalence in the population of 20 – 60% with important impact on supportive laboratory results. In the present study we look at all patients referred to borrelia antibody assays during 2010 - 2014 and follow the procedure until final diagnosis of NB.

### **Methods**

A derivation cohort consisting of 6530 consecutive patients during 2010 – 2013, referred for borrelia serology and for further evaluation of suspected disseminated borreliosis, was studied. In an aliquot of 77 patients a Csf-biomarker panel consisting of cytokines and C1q were studied in relation to the diagnosis. From the data a diagnostic algorithm was constructed. The algorithm performance was studied in a validation cohort of 3370 consecutive patients during 2013 – 2014.

Serum C6-peptide IgM/IgG antibodies were quantitated by C6 Lyme ELISA Kit, Immunetics, USA. IgG-antibodies to a panel of recombinant borrelia antigens were screened by an ELISA (recomWell, Mikrogen, Germany) and verified by a multiplexed particle method for Luminex RecombBead, Mikrogen, Germany). CSF IgG-ABI was determined by multiplexed particle method and the i.t. specific antibody production index (ABI) was automatically calculated from CSF and serum albumin and IgG concentrations and from the specific antibody findings in CSF and serum according to Reiber. CSF-CXCL13 was measured using ELISA (Quantikine, R&D Systems, USA) according to the instructions supplied by the manufacturer.

CSF-C1q was assayed by ELISA (Platinum ELISA®, eBioscience, USA) with a detection limit of 0.08 ng/ml. Cytokines and chemokines IL-1 $\beta$ , 2, 4, 5, 6, 7, 8 (CXCL8), 10, 12p70, IL-13, IFN- $\gamma$ , GM-CSF, MCP-1 (CCL2) and TNF- $\alpha$ , were measured in CSF by a multiplexed kit for the Luminex platform (Milliplex® Map Human Cytokine/Chemokine - Premixed 14 Plex, Millipore, USA).

Additionally routine laboratory methods were applied for blood and Csf-studies. Diagnostic performance parameters and likelihood ratios were calculated by ROC-analysis and for i.t. ABI by a multilevel categorical test. Criteria for case definition were initially those of EFNS. A stepwise probabilistic diagnostic algorithm is proposed.

### **Results.**

In the derivation cohort a clinical possibility of NB motivating investigation of the cerebrospinal fluid (Csf) was found in 533 (8.2%) patients. In these patients 90 (16.9%) definite NB, 216 (40.5%) possible NB and 227 (42.6%) non-NB were found by EFNS case definition.

By combining the data with Csf-CXCL13 as activity marker, the corresponding figures were 77 (14.4%) NB, no possible NB and 456 (85.6%) non-NB. These figures were strongly supported by further biomarker analysis.

In the validation cohort Csf samples were obtained from 154 (4.6%), 20 (13%) were definite NB, no possible NB. There are other causes of lymphocytic pleocytosis and positive antibody indices than neuroborreliosis and the frequency of NB even in a high risk population is low. The probabilistic diagnostic calculations will be presented.

### **Conclusions.**

A reliable diagnosis of NB cannot rely upon the EFNS guidelines. The category of possible NB must be eliminated.