

# Detection of antigen-specific T cells in patients with neuroborreliosis

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## ABSTRACT

There is a lack of laboratory tests in clinical practice that can detect the activity of borrelial infection. This was the reason for testing an antigen-specific T-cell detection method in patients with neuroborreliosis: the ELISPOT method, which is capable of detecting antigen-specific T lymphocytes in clinical conditions. A group of 32 patients (20 diagnosed with neuroborreliosis; NB) was examined using this commercial method (LymeSpot intrerferon- $\gamma$  Assay Kit®). Of these 20 NB patients, 10 were found to be positive and 10 negative; four of the five persons tested prior to the antibiotic treatment were positive. Eight patients served as the control group, giving four positive and four negative

results. The results achieved so far appear to be unequivocal; still, the test could be expected:

- a) in non-specific clinical symptoms with borderline or negative proof of antibodies;
  - b) in early stage of the disease;
  - c) in the case of seropositivity and unequivocal clinical picture.
- The basic prerequisite for the clinical utilization of the method, however, is that it be thoroughly tested.

## KEYWORDS:

neuroborreliosis – immune response – T cells – interferon- $\gamma$

## SOUHRN

**Pícha D., Moravcová L., Smíšková D.: Průkaz antigenně specifických T lymfocytů u pacientů s neuroborreliózou**

V klinické praxi chybí laboratorní testy, které prokazují aktivitu borreliové infekce. Z tohoto důvodu byla testována metoda průkazu antigenně specifických T lymfocytů u skupiny pacientů s neuroborreliózou (LymeSpot Assay Kit®). Celkem bylo vyšetřeno 32 pacientů. U 20 pacientů s neuroborreliózou (NB) bylo získáno 10 pozitivních a 10 negativních výsledků, z 5 pacientů vyšetřených před léčbou byli 4 pozitivní. U 8 kontrolních pacientů s infekcemi neborreliové etiologie byli 4 pacienti pozitivní a 4 negativní. Dosavadní výsledky jsou

nejednoznačné, přesto lze předpokládat diagnostický přínos u NB při:

- a) nespecifickém klinickém obrazu s hraničním nebo negativním průkazem protilátek,
- b) v raném stadiu onemocnění,
- c) v případě séropozitivity a nejednoznačného klinického obrazu.

Podmínkou využití metody je však její důsledné otestování.

## KLÍČOVÁ SLOVA:

neuroborrelióza – imunitní odpověď – T lymfocyty – interferon- $\gamma$

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## INTRODUCTION

Lyme borreliosis (LB) is a spirochetal disease affecting a number of organs, most frequently the nervous system, skin and joints. The clinical symptoms associated with LB vary considerably and serology therefore provides the main diagnostic support. Frequent nonspecific reactions necessitate the second step – confirmation by WB. Unfortunately, the sensitivity of many serological tests hardly exceeds 50% in early forms, irrespective of whether native antigens, a mixture of recombinant antigens or specific peptides are used [1]. On the contrary, antibodies can persist for months or years after successful antibiotic treatment [2] and seronegative forms of LB have also been described [3, 4]. The other factors make the antibody proof diagnostically inferior. A positive serological test in itself is not confirmation of an active

infection [5]; some cross-reactive antigen reactions do exist among bacterial pathogens (*Treponema pallidum* e.g.), and isolated IgM positivity can be a symptom of polyclonal immune response activation (Epstein-Barr virus infection) or rheumatic diseases. The solitary detection of specific antibodies in all of these cases is not sufficient for the diagnosis of LB and antibiotic treatment. Consequently, other methods are tested to improve the laboratory LB diagnostic. T lymphocytes are necessary for cell-mediated immunity to function properly. Shortly after the initiation of the infectious process, antigen-specific T lymphocytes are stimulated for proliferation, differentiation and cytokine secretion. These changes lead to the synthesis of B lymphocytes and the production of specific antibodies. After the introduction or start of the immune reactions, the intensity of antigen stimulation decreases and, hand in hand with this, there is a rapid

decrease in the number of activated T-cells and cytokine levels. For this reason, the monitoring of the activated T-cells is tested as an additional laboratory method to support standard serological laboratory tests. Compared with the latter, the great advantage of cellular immune response tests is that they provide more accurate information on recent immunologic response. Some studies describing borrelia-specific T-cells tests have already been published [for instance 6, 7, 8] and their results suggest they could be helpful for early LB diagnostics. However, this experience has not been systemized or fully evaluated. Our first cases of proving antigen-specific T-cells in LB are published in this article.

## PATIENTS GROUP AND METHODS

Thirty patients hospitalized with a suspected neuroinfectious disease were included in this prospective study. CSF was examined in all these cases; two additional patients had other infections (bacterial pneumonia, pulmonary TBC). The group studied was divided into three parts. Group A – 20 patients with NB, all these patients were positive for intrathecal synthesis of antiborrelial antibodies (positive  $AI_{bb}$ ), and in 14 of them the CSF lymphocytic pleocytosis was proven. Group B – 4 patients with CSF and serum LB antibody positivity,  $AI_{bb}$  negative, one with CSF pleocytosis; Group C – 8 control patients, 6 patients with aseptic CNS infection (3 with tick-borne encephalitis – TBE, 3 with unknown etiology), 1 with pneumonia, 1 with pulmonary TBC. Seven out of 20 patients with positive  $AI_{bb}$  were examined before the antibiotic treatment was initiated, and the rest during therapy.

Antiborrelial antibodies were detected using the commercial diagnostic kit ELISA-Viditest anti-borrelia recombinant IgG VlsE/IgM (VIDIA s. r. o., ČR) and the results were confirmed by Western blotting (Blot-Line Borrelia HGAIgM/HGAIG TestLine, Clinical diagnostic s. r. o., ČR).

Intrathecal synthesis of antiborrelial antibodies ( $AI_{bb}$ ) was calculated using the VidiTab computer program (Vidia s.r.o., ČR); the value  $AI_{bb} \geq 1.5$  was considered positive.

A borrelia-specific T lymphocytes examination was performed using the commercial LymeSpot Assay Interferon- $\gamma$  Kit (GenID GmbH, Germany, imported into the Czech Republic by Medisko Praha, s. r. o.) at the manufacturer's recommendation. Peripheral blood lymphocytes were isolated from fresh blood in gradient Ficoll-Paque™ PLUS and the concentration was adjusted to  $2 \times 10^6$  cells/mL. This suspension was added to wells coated with an anti-human interferon- $\gamma$  monoclonal antibody. The specific borrelial antigen *Borrelia burgdorferi* B31 lysate and the mixture of recombinant OspC and OspA with p18 peptide were added and then incubated for 24 hours in a CO<sub>2</sub> incubator. During this period, the synthesized interferon- $\gamma$  was bound by the monoclonal antibody. After removing the cell suspension, the secondary anti-human interferon- $\gamma$  alkaline phosphatase labelled antibody was added. After the following washing steps the interferon- $\gamma$  production was visualized by the addition of the substrate solution (BCI/NBT) and the colour reaction was read as a spot at the bottom of the well. One spot was identified as one cell producing

Table 1. Results of LymeSpot interferon- $\gamma$  Assay Kit

Group	Negative SI < 5	Positive SI > 5	Number
A	10	10	20
B	4	0	4
C	4	4	8
Number	18	14	32

interferon- $\gamma$  and the results were counted using the EliSpot automatic reader (AOD iSPOT, AID, Germany). The stimulation index (SI) is used for interpretation. SI expresses the number of the spots from the culture with the antigen mixtures/number of spots from the negative control. The value of the SI > 5 was interpreted as positive.

## RESULTS AND DISCUSSION

The results of examination of the antigen-specific effector T<sub>H</sub> cells are summarized in Table 1. In group A, the result was positive in 10 patients; 10 results were negative. However, one difference was found when the results were assessed and compared with the beginning of the antibiotic treatment. Of the five patients with the positive AI and CSF pleocytosis examined before treatment, four were positive and only one negative. On the contrary, when they were tested during antibiotic treatment (9 patients), seven were negative and only two positive. Of the 6 patients with the positive  $AI_{bb}$  without CSF pleocytosis, 4 were positive and 2 negative. In group B (4 patients;  $AI_{bb}$  negative), no positive result was found. An interesting result was discovered in the control group (C) – four positives (one patient suffered from TBE and three from aseptic meningoencephalitis. Two had no circumstance evidence leading to suspicion of NB, one was treated by ceftriaxone due to IgM seropositivity for LB discover later. No later IgG seroconversion was observed. It is evident that the number of patients examined is far from sufficient for a detailed assessment or statistical analysis. The results presented herein may indicate that future testing should first be targeted at a more homogenous cohort, although a study carried out with a bigger group could certainly provide some very interesting results as well. The positive results in the group C can serve as an example. Moreover, the time factor seems to be important. It should be explained whether the higher number of positive results before treatment is an accidental fluctuation or an important phenomenon, while the latter has already been published [6, 8]. But it is necessary to verify whether ELISPOT positivity – proof of interferon- $\gamma$  synthesis after the stimulation of the borrelial antigens – is only specific to the borrelial infection. The control group should comprise healthy patients and patients with other infectious diseases in an acute or delayed phase of the disease as well, especially those whose clinical symptoms share clinical manifestations of LB. Laboratory tests based on the proof of cytokines are rarely a part of routine laboratory diagnostic either in LB or other infections (exceptions exist, e.g. the QuantiFERON test, T-SPOT TB) [9]. The advantage of cellular tests based on the immunochemical principle (such as ELISPOT) is that they save time – 24 hours with ELISPOT, 6 days with

## KRÁTKÉ SDĚLENÍ

proliferative radionuclide-based methods. Moreover, these methods are relatively risky due to the use of radionuclide material and the possible limits on repetition. The test results based on the examination of the whole blood (like QuantiFERON test) can be influenced by the production of cytokines other than interferon- $\gamma$  that are secreted by other cells (natural killer cells, monocytes), which results in false positivity. The disadvantage of ELISPOT tests is that their standardization can sometimes be limited as some methodological factors can interfere – e.g. the taking and preparation of lymphocytes (temperature, transportation, treatment etc.), which to some extent complicate routine clinical practice. Despite the aforementioned problems, we consider the LymeSpotAssay interferon- $\gamma$  Kit a possible contribution for additional LB diagnostic especially in specialized centres when:

- i) clinical symptoms are not diagnostic and a serological test is borderline or negative,
- ii) in long-term diseases with a non-specific clinical picture and seropositivity,
- iii) in the early phase of infection (2–6 weeks).

Besides the experimental utilization of the method, a necessary but not a *sine qua non* prerequisite is careful clinical testing, a condition which, it seems, has not so far been fulfilled.

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