Antibody Response In *Borrelia Miyamotoi* Infection Studied By Protein Microarray

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Number of cases of different tick-born infections (data obtained by Federal service for supervision of consumer rights protection and human well-being in 2017)

Total number of registered in Russia in 2017 tick bites 509262

- TBE 1943 cases
- Tick-borne rickettsiosis 1984 cases
- Anaplasmosis 31 cases
- Erlichiosis 19 cases
- CCHF 79 cases

No disease or unknown, 98%
Structure of registered tick-born infections (data obtained by Federal service for supervision of consumer rights protection and human well-being in 2017)

- TBE
- Tick-born rickettsiosis
- Anaplasmosis
- Erlichiosis
- CCHF
- Lyme disease

Lyme disease, 62.35%
TBE, 18.04%
Tick-born rickettsiosis, 18.42%
Erlichiosis, 0.18%
Anaplasmosis, 0.29%
CCHF, 0.73%
Two-step protocol of Lyme disease diagnostics

1\textsuperscript{st} step ELISA

- Antibodies to different antigens
- High specificity
- Low cost
- Automation
- High sensitivity
- Bacterial lysate or individual recombinant antigen
- Different wells for IgG/IgM

2\textsuperscript{nd} step Immunoblot

- Antibodies to different antigens
- High specificity
- High cost
- Two strips for IgG/IgM
- Often visual result interpretation
Protein Microarray

- one-step analysis for multiple markers
- very high informatively of test
- possibility to produce screening and confirmation tests in the same time

As sensitive as ELISA, as specific as blot
Manufacturing of the microarrays

Spotting of recombinant proteins and control solutions in triplicates

Procedure of analysis using the microarray based kit

Add sample + dilution 1 to 9 (5мин)
Incubation (30мин)

Scanning, quantitation (15мин)
Washing (5мин)

Add conjugate mixture (Cy3-anti IgM + Cy5-anti IgG, incubation(30 min )
Washing (5мин)

Aldehyde-activated glass-slides (VALS-25, Cell)
12 arrays on each slide

IgM and IgG id detecting in one well but separately
Microarray for Lyme disease antibody detection

Concentrations of anti-Borrelia - specific IgM and IgG to individual proteins are interpolated from the human IgM and human IgG standard curves using ImStar software. Specific IgM and IgG levels are considered significant if they exceeded 5 ug/ml

**Interpretation rules for microarray (IgG)**

<table>
<thead>
<tr>
<th><strong>Antibodies</strong></th>
<th><strong>Result</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative result</td>
<td>1. No antibodies to all <em>Borrelia afzelii</em> &amp; <em>Borrelia garinii</em> antigens; 2. Antibodies found only to p17 proteins.</td>
</tr>
<tr>
<td>Positive result</td>
<td>1. Antibodies found to all <em>Borrelia afzelii</em> &amp; <em>Borrelia garinii</em> antigens; 2. Antibodies found to VlsE antigens; 3. Antibodies found to two groups of antigens from: p100, p41, p17, OspC, p58, Bbk32, p39.</td>
</tr>
<tr>
<td>Equivocal result</td>
<td>Antibodies found to one group of antigens from: p100, p41, OspC, p58, Bbk32, p39</td>
</tr>
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**Interpretation rules for microarray (IgM)**

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<thead>
<tr>
<th><strong>Antibodies</strong></th>
<th><strong>Result</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative result</td>
<td>1. No antibodies to all <em>Borrelia afzelii</em> &amp; <em>Borrelia garinii</em> antigens; 2. Antibodies found only to p100 proteins</td>
</tr>
<tr>
<td>Positive result</td>
<td>1. Antibodies found to all <em>Borrelia afzelii</em> &amp; <em>Borrelia garinii</em> antigens; 2. Antibodies found to OspC antigens; 3. Antibodies found to two groups of antigens from: p41, p17, VlsE.</td>
</tr>
<tr>
<td>Equivocal result</td>
<td>Antibodies found to one group of antigens from: p41, VlsE, p17 (with or without combination with antibodies to: p58, p39, BBK32 antigens)</td>
</tr>
</tbody>
</table>
Evaluation of developed microarray kit

Diagnostic sensitivity

<table>
<thead>
<tr>
<th>LD stage</th>
<th>IgM</th>
<th>IgG</th>
<th>IgM &amp;/or IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
<td>equivocal</td>
<td>positive</td>
</tr>
<tr>
<td>I (n=79)</td>
<td>56,8%</td>
<td>11,8%</td>
<td>47,1%</td>
</tr>
<tr>
<td>II (n=120)</td>
<td>60,0%</td>
<td>3,6%</td>
<td>78,8%</td>
</tr>
<tr>
<td>III (n=81)</td>
<td>35,7%</td>
<td>5,4%</td>
<td>96,4%</td>
</tr>
</tbody>
</table>

Diagnostic specificity (n=300) – 97%
In year 2003-2004 Borrelia miyamotoi DNA was found in blood of 40% of patients with Lyme disease without erythema migrans.

DNA of Borrelia miyamotoi could be found in from 0.5% to 15% of ticks, collected in USA, Sweden, Germany, France, Poland, Russia.

To 2018 DNA of Borrelia miyamotoi was found in the blood samples from patients with fever appeared after tick bite in Kirov, Sankt-Petersburg, Ijevsk, Cherepovec, Ekaterinburg, Tomsk, Omsk, Novosibirsk, Krasnoyarsk regions of Russia.
B. miyamotoi taxonomy

Tick-born relapsing fever

Lyme disease
Clinic evidence of tick-born relapsing fever, etiologically connected with *B. miyamotoi*

**Incubation period:**
In average **14 days** (10-21 days) after tick bite

**Symptoms (duration 3 – 7 days):**
- fever up to 40°C (100%)
- sweating (100%)
- chill (100%)
- weakness (100%)
- headache (100%)
- dizziness (90%)
- myalgia, arthralgia (54%)
- nausea (66%)
- absence of erythema migrans (98%)

**General tests:**
- Thrombocytopenia (46%), without an increase in capillary fragility
- Increase of ALT/AST (61%)
- Symptoms of renal impairment (MAU, proteinuria, erythrocyturia) (20%)

**Without treatment**
- 2\textsuperscript{nd} and 3\textsuperscript{d} waves of fever is possible
In vitro diagnostics of tick-born relapsing fever, etiologically connected with *B. miyamotoi*

**Direct methods**
- Microscopy
  - Dark field microscopy
  - Viewing in a phase-contrast microscope
  - Thick drop method
- Cultivation
  - Special procedures for the blood collection
  - Special procedures for sample preparation
  - Special culture media is needed
- IFA
- PCR

**Indirect methods** - identification of antibodies to antigens of the pathogen

Determination of *B.miyamottii* in the bacterial fraction of the patient’s blood plasma, in which the DNA of the pathogen was detected by PCR, by the modified “thick drop” method.

Yekaterinburg, 2011

The Open Microbiology Journal, 2008, 2, 10-12
Development of the microarray kit for detection of antibodies to antigens of Borrelia Miyamotoi

B. miyamotoi expresses some common antigens with c B. burgdorferi s.l. :

- **FlaB (p41)** - structure protein of flagella, highly antigenic, non-specific, marker of early IgM response
- **GroEL** – heat-shock protein, highly antigenic, non-specific
- **p66** - adhesin, highly antigenic
- **BmpA (p39)** - membrane protein, highly specific, weakly antigenic

B. miyamotoi expresses antigen, which is not present in B. burgdorferi s.l.

- **GlpQ** - glycerophosphodiester phosphodiesterase, highly antigenic, highly specific for tick-born relapsing fever group,

**Candidate proteins-antigens** for differential diagnosis of the disease caused by B. miyamotoi

- **Vlp15/16, Vlp18, Vsp1 и Vlp5** - variable surface proteins, highly antigenic
Microarray for Lyme disease & B. miyamotoi antibody detection
Clinical samples

Patients in which blood sample, taken at admission, B.miyamotii DNA were identified
- fixed tick bite during last month
- fever, headache, chills, weakness, sweating, myalgia or arthralgia were present
- in blood sample, taken at admission, B.miyamotii DNA was identified
- the absence of erythema migrans
- laboratory excluded TBE, Lyme disease

Group 1. n = 24 (MO "New hospital", Ekaterinburg)
Blood samples taken:
0 - at admission (3±1,5) days after symptoms onset (n = 24)
1 – 1 -4 samples taken between days 1 and 10 of hospitalization (n = 59)
2 – day 10 -20 after symptoms onset (n = 13)
3 – day 60 -90 after symptoms onset (n = 15)
4 - day 120-180 after symptoms onset (n = 13)
5 - day 210-290 after symptoms onset (n = 15)

Group 2. n = 26 (ISMA, Izhevsk)
Blood samples taken :
0 - at admission (3±1,5) days after symptoms onset (n = 26)
1 – day 5-17 after symptoms onset (n = 23)
2 – day 30-45 after symptoms onset (n = 12)
3 - day 90-125 after symptoms onset (n = 7)
4 – day 340-365 after symptoms onset (n =5)

Group 3 Healthy controls from endemic territory (n = 61)
Detection of IgM and IgG antibodies specific to antigens of *B. miyamotoi* and *B. burgdorferi* s.l., in the serum samples of patients

<table>
<thead>
<tr>
<th>Protein</th>
<th>GlpQ</th>
<th>Vsp1</th>
<th>Vlp5</th>
<th>Vlp15/16</th>
<th>Vlp18</th>
<th>B.b.s.l.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM pos/total</td>
<td>19/24</td>
<td>12/24</td>
<td>10/24</td>
<td>15/24</td>
<td>4/27</td>
<td>19/24</td>
</tr>
<tr>
<td>IgM appearance</td>
<td>Day 6</td>
<td>Day 6</td>
<td>Day 7</td>
<td>Day 6</td>
<td>Day 49</td>
<td>Day 7</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM pos/total</td>
<td>23/26</td>
<td>6/26</td>
<td>11/26</td>
<td>13/26</td>
<td>3/26</td>
<td>13/26</td>
</tr>
<tr>
<td>IgM appearance</td>
<td>Day 9</td>
<td>Day 9</td>
<td>Day 7</td>
<td>Day 7</td>
<td>Day 7</td>
<td>Day 7</td>
</tr>
</tbody>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG pos/total</td>
<td>21/24</td>
<td>4/24</td>
<td>13/24</td>
<td>18/24</td>
<td>2/24</td>
<td>3/24</td>
</tr>
<tr>
<td>IgG appearance</td>
<td>73 day</td>
<td>73 day</td>
<td>63 day</td>
<td>79 day</td>
<td>20 day</td>
<td>73 day</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG pos/total</td>
<td>16/26</td>
<td>7/26</td>
<td>10/26</td>
<td>14/26</td>
<td>2/26</td>
<td>4/26</td>
</tr>
<tr>
<td>IgG appearance</td>
<td>29 day</td>
<td>30 day</td>
<td>10 day</td>
<td>16 day</td>
<td>30 day</td>
<td>25 day</td>
</tr>
</tbody>
</table>
Seroconversion of antibodies specific to antigens of B. miyamotoi and B. burgdorferi s.l. in blood serum samples of patients during hospitalization

<table>
<thead>
<tr>
<th>Day after symptoms onset</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>1-3</td>
<td>0/24</td>
<td>0/24</td>
</tr>
<tr>
<td>6-9</td>
<td>16/24</td>
<td>4/24</td>
</tr>
<tr>
<td>10-14</td>
<td>4/24</td>
<td>2/24</td>
</tr>
<tr>
<td>∑, 6-14 day</td>
<td>20/24</td>
<td>6/24</td>
</tr>
<tr>
<td>∑, seroconversion</td>
<td>21/24</td>
<td></td>
</tr>
</tbody>
</table>

* The average duration of hospitalization for a disease etiologically associated with B. miyamotoi is 10 days.
Detection of GlpQ-specific antibodies in blood serum samples of patients for the entire follow-up period (n=222)

One arbitrary unit (AU) corresponds to 1 µg/ml of IgM or IgG antibody
Detection of VMPs-specific antibodies in blood serum samples of patients for the entire follow-up period (n=222)
Detection of antibodies specific to the antigens of B. miyamotoi and B. burgdorferi s.l. in blood serum samples of patients for the entire follow-up period

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GlpQ</td>
<td>20/27</td>
<td>23/26</td>
<td>1/61</td>
</tr>
<tr>
<td>Two Vlp/Vsp without Ab to GlpQ</td>
<td>3/27</td>
<td>1/26</td>
<td>-</td>
</tr>
<tr>
<td>B.b.s.l. without Ab to GlpQ &amp; Vlp/Vsp</td>
<td>1/27</td>
<td>1/26</td>
<td>1/61</td>
</tr>
<tr>
<td>IgG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GlpQ</td>
<td>23/27</td>
<td>16/26</td>
<td>4/61</td>
</tr>
<tr>
<td>Two Vlp/Vsp without Ab to GlpQ</td>
<td>2/27</td>
<td>1/27</td>
<td>-</td>
</tr>
<tr>
<td>B.b.s.l. without Ab to GlpQ &amp; Vlp/Vsp</td>
<td>-</td>
<td>-</td>
<td>2/61</td>
</tr>
<tr>
<td>IgM&amp;IgG to GlpQ/two Vlp/Vsp</td>
<td>21/27</td>
<td>18/26</td>
<td>-</td>
</tr>
<tr>
<td>IgM/IgG to GlpQ/twoVlp/Vsp</td>
<td>26/27</td>
<td>24/26</td>
<td>5/61</td>
</tr>
<tr>
<td>IgM/IgG to GlpQ/two Vlp/Vsp/ B.b.s.l.</td>
<td>27/27</td>
<td>25/26</td>
<td>8/61</td>
</tr>
</tbody>
</table>
The concentration of protein-specific antibodies to B. miyamotii antigens, in healthy donor groups (n=70) and 50 patients with ICD, determined by B. miyamotii. Any VMPs is the highest value of the 4 VMP used for a particular sample. Data for patients with B.miyamotii-induced ICD were obtained at optimal timing of IgM and IgG detection, between 7-50 and 21-200 days from the onset of symptoms, respectively. A red line indicates the selected threshold for separating positive and background signals.
Conclusion

• The seroconversion of specific to proteins-antigens of B. Miyamotoi IgG and IgM is shown in serum samples of patients in which blood sample, taken at admission, B.miyamotoi DNA were identified.

• During the hospitalization period (6-10 days after the onset of symptoms) seroconversion was detected in 80% and 90% of the studied paired blood serum samples.

• The median time to detect IgM to antigens of B.miyamotoi is 6-9 days after the onset of symptoms.

• The median time to detect IgG to antigens of B.miyamotoi is 20-30 days after onset of symptoms.

• GlpQ and Vlp15/16 is one of the highest diagnostic sensitivity of the studied antigens.

• It is possible to propose the following algorithm of laboratory confirmation of cases of the disease, etiologically associated with B.miyamotoi:
  1. On the day of the onset of symptoms - determination of the pathogen DNA in the bacterial fraction of the blood, determination of antibodies to antigens of the pathogen.
  2. 7-10 days after the onset of symptoms - determination of antibodies to pathogen proteins, confirmation of IgM seroconversion.
  3. 30-45 days after the onset of symptoms - the detection of antibodies to the proteins-antigens of the pathogen, confirmation of seroconversion of IgG (if possible).
Thank you for your attention!

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University of Amsterdam, Academic Medical Center, Amsterdam, Nederland
J. Koetsveld, J. Hovius
Important antigens of *B. burgdorferi* s.l., used in serodiagnosis of Lyme disease

**p100** – protein of membrane vesicles, highly antigenic, highly specific

**VlsE** – surface lipoprotein (Variable major protein-like sequence), its highly specific IR6 domain is often used in ELISA

**p58** - membrane protein, highly antigenic.

**p41 & p41int** – structure protein of flagella, highly antigenic, non-specific, marker of early IgM response

**p39=BmpA** - membrane protein, highly specific, weakly antigenic

**BbK32** – fibronectin binding membrane protein, highly antigenic, highly specific

**OspC** – membrane lipoprotein, highly antigenic, highly specific, marker of early IgM response

**p17=DbpA** (Decorin-binding protein A) – highly antigenic, highly specific.