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

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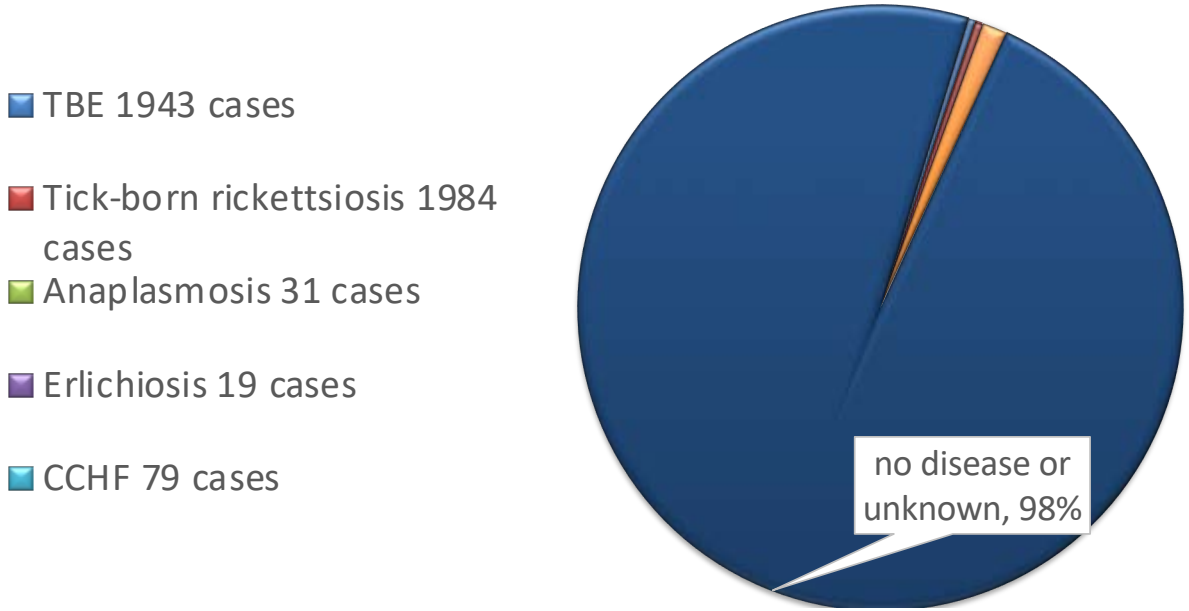
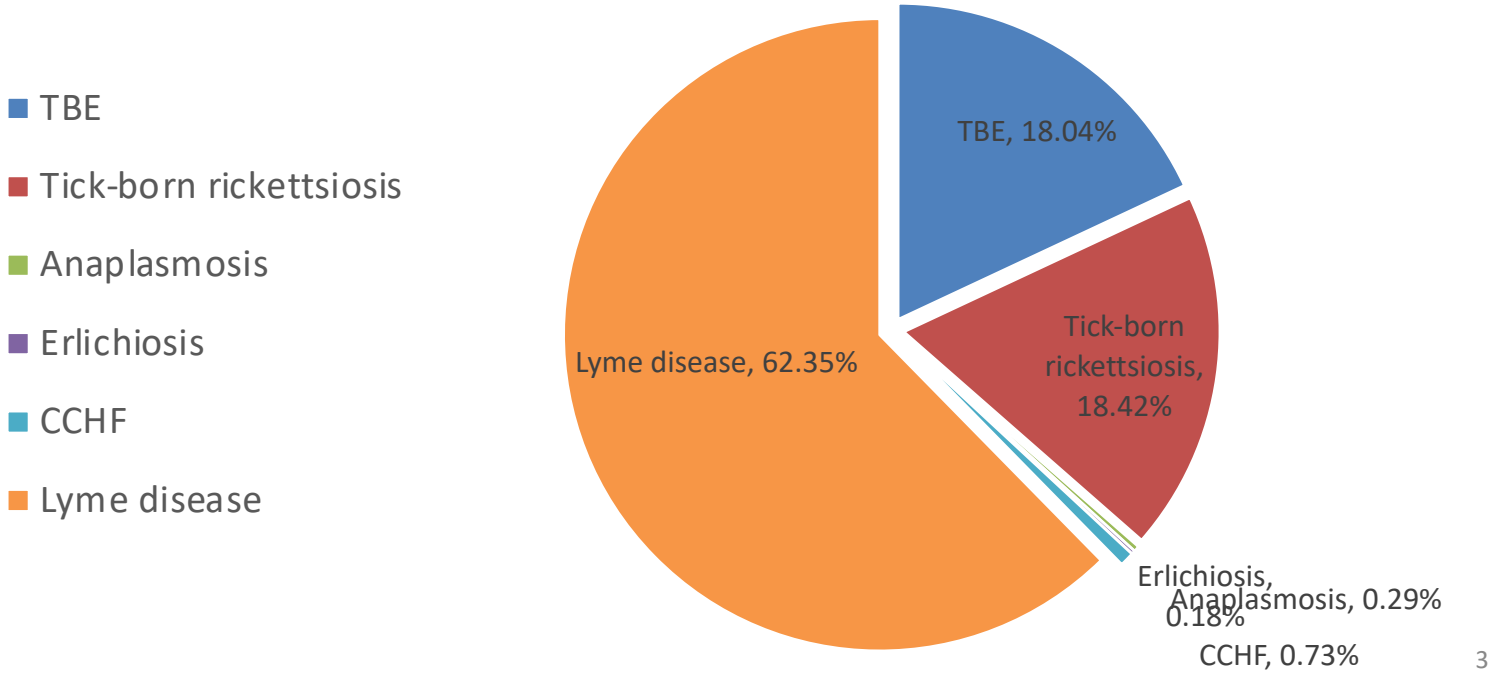
Number of cases of different tick-born infections (data obtained by Federal service for supervision of consumer rights protection and human well-being in 2017)

Total number of registered in Russia in 2017 tick bites 509262

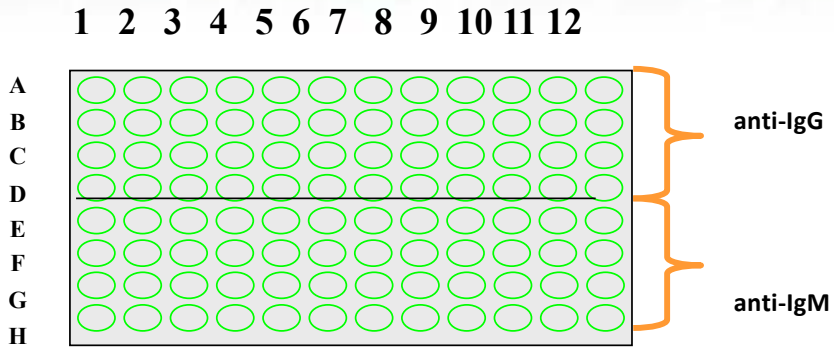


Structure of registered tick-born infections (data obtained by Federal service for supervision of consumer rights protection and human well-being in 2017)



Two-step protocol of Lyme disease diagnostics

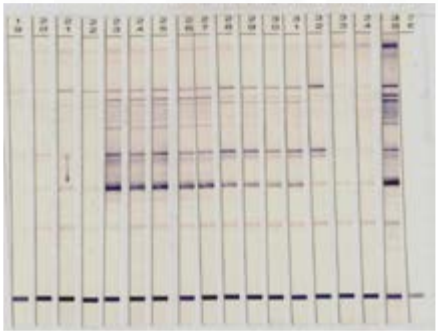
1st step ELISA



- Low cost
- Automation
- High sensitivity

- Bacterial lysate or individual recombinant antigen
- Different wells for IgG/IgM

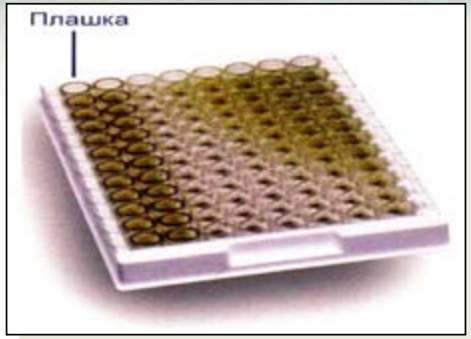
2nd step Immunoblot



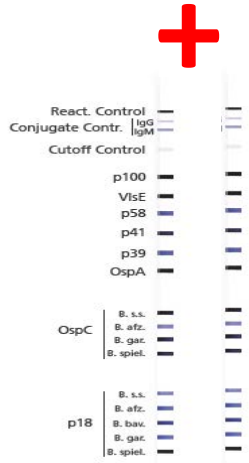
- Antibodies to different antigens
- High specificity

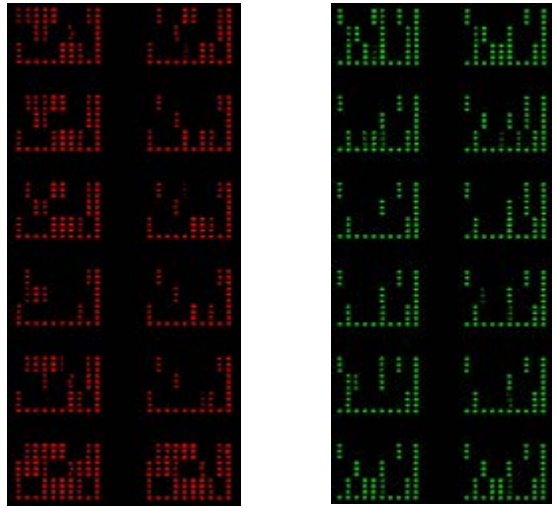
- High cost
- Two strips for IgG/IgM
- Often visual result interpritation

Protein Microarray



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

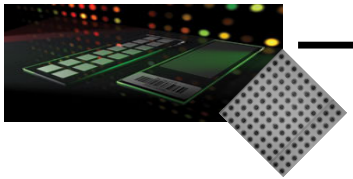




As sensitive as ELISA, as specific as blot

Manufacturing of the microarrays

Spotting of recombinant proteins and control solutions in triplicates



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

Procedure of analysis using the microarray based kit

Add sample + dilution 1 to 9 (5мин)

Incubation (30min)

Washing (5мин)

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

Washing (5мин)

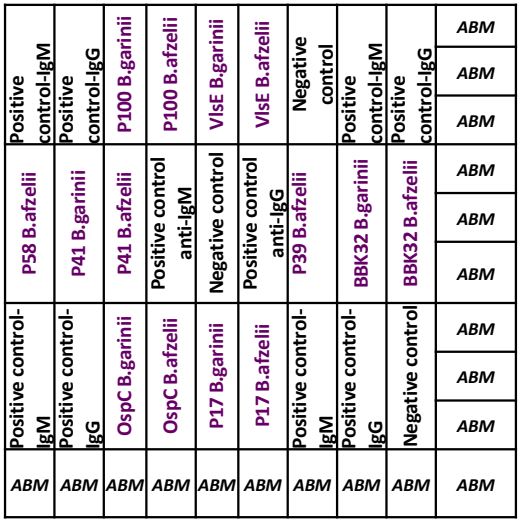
Scanning, quantitation (15мин)

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



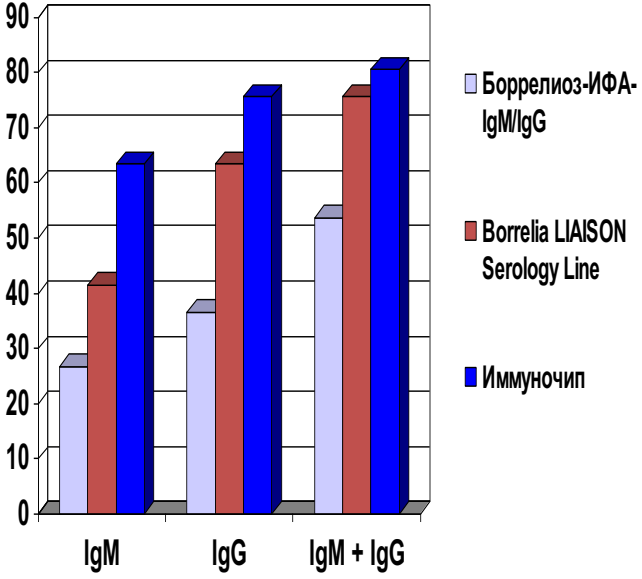
Interpretation rules for microarray (IgG)

Negative result	1.No antibodies to all *Borrelia afzelii* & *Borrelia garinii* antigens; 2.Antibodies found only to p17 proteins.
Positive result	1.Antibodies found to all *Borrelia afzelii* & *Borrelia garinii* antigens; 2.Antibodies found to VlsE antigens; 3.Antibodies found to two groups of antigens from: p100, p41, p17, OspC, p58, Bbk32, p39.
Equivocal result	Antibodies found to one group of antigens from: p100, p41, OspC, p58, Bbk32, p39

Interpretation rules for microarray (IgM)

Negative result	1.No antibodies to all *Borrelia afzelii* & *Borrelia garinii* antigens 2.Antibodies found only to p100 proteins
Positive result	1.Antibodies found to all *Borrelia afzelii* & *Borrelia garinii* antigens; 2.Antibodies found to OspC antigens; 3.Antibodies found to two groups of antigens from: p41, p17, VlsE.
Equivocal result	Antibodies found to one group of antigens from: p41, VlsE, p17 (with or without combination with antibodies to: p58, p39, BBK32 antigens)

Evaluation of developed microarray kit



(n=42)

Diagnostic sensitivity

LD stage	IgM		IgG		IgM &/or IgG
	positive	equivocal	positive	equivocal	
I (n=79)	56,8%	11,8%	47,1%	16,7%	70,4%
II (n=120)	60,0%	3,6%	78,8%	9,1%	92,7%
III (n=81)	35,7%	5,4%	96,4%	1,8%	100%

Diagnostic specificity *(n=300) – 97%*

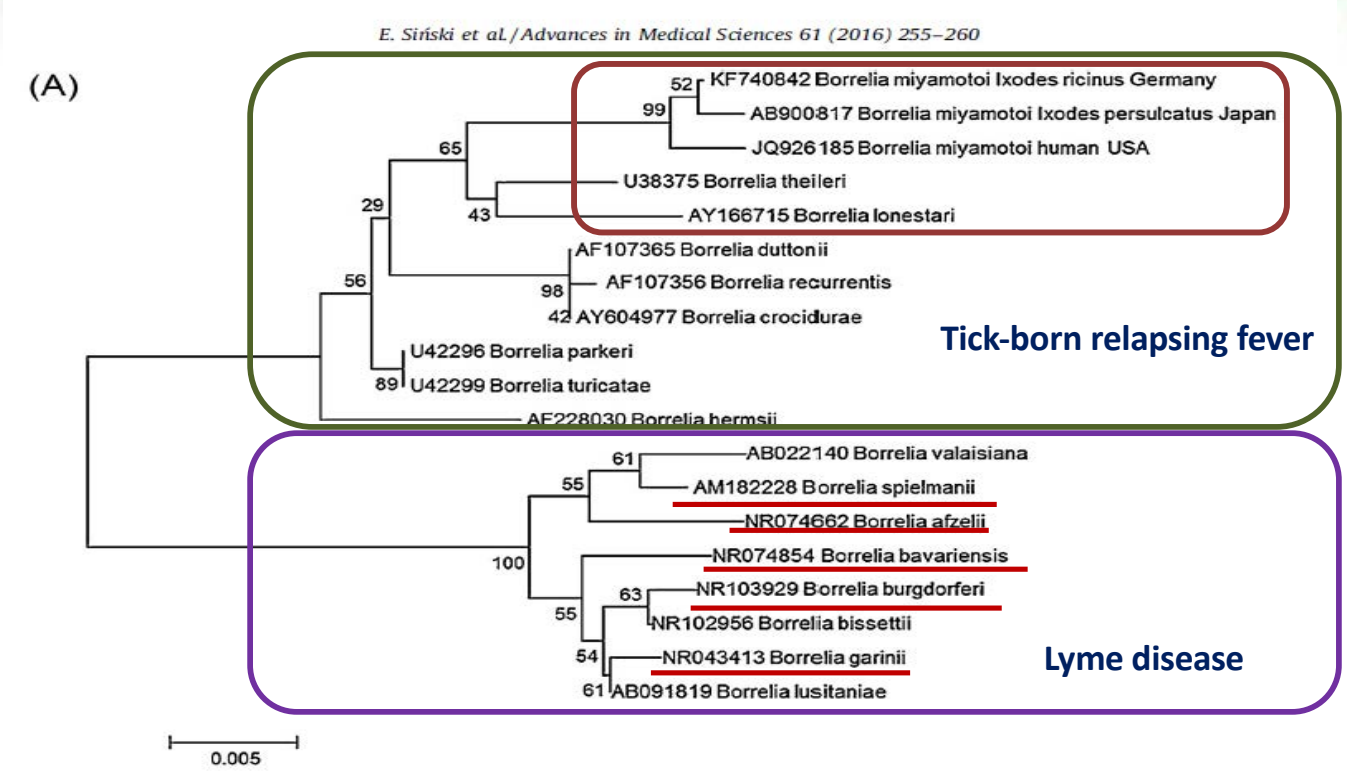
Tick-born borreliosis, etiologically connected with *B. miyamotoi*

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



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

- **2nd and 3d 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**

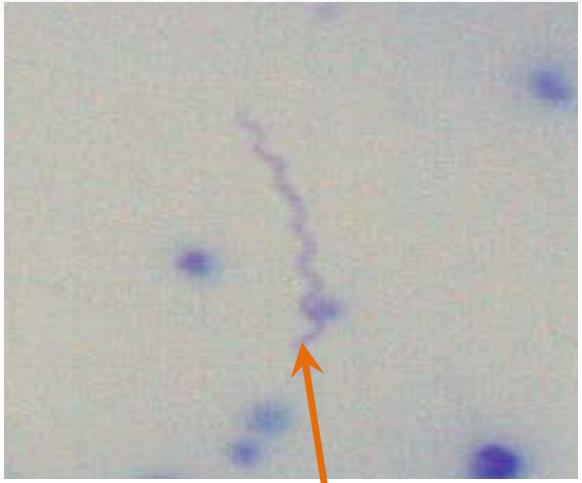
- **Cultivation**

✓ **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.miyamotoi 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

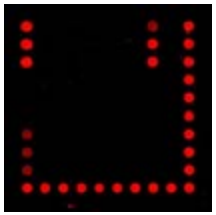
B. miyamotoi expresses antigen, which is not present in B. burgdorferi s.l.

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

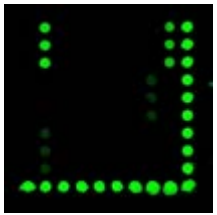
Candidate proteins-antigens for differential diagnosis of the disease caused by B. miyamotoi
VMPs - Vlp15/16, Vlp18, Vsp1 и Vlp5- variable surface proteins, highly antigenic

Microarray for Lyme disease & B. miyamotoi antibody detection

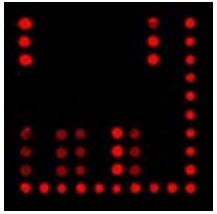
IgG profile, negative sample



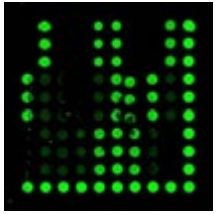
IgM profile, negative sample



IgG profile, positive sample



IgM profile, positive sample



IgG, 5uf/ml	P41 B.garinii	IgG, 50uf/ml	ABM	ABM	ABM	ABM	ABM	ABM	ABM
IgM, 5ug/ml	P41 B.afzelii	IgM, 50ug/ml	ABM	ABM	ABM	ABM	ABM	ABM	ABM
GlpQ v1 B.miyamotoi	P39 B.afzelii	P100 B.garinii	ABM	ABM	ABM	ABM	ABM	ABM	ABM
GlpQ v2 B.miyamotoi	BBK32 B.garinii	P100 B.afzelii	ABM	ABM	ABM	ABM	ABM	ABM	ABM
Vsp1 B.miyamotoi	BBK32 B.afzelii	VlsE B.garinii	ABM	ABM	ABM	ABM	ABM	ABM	ABM
Vlp5 B.miyamotoi	OspC B.garinii	VlsE B.afzelii	ABM	ABM	ABM	ABM	ABM	ABM	ABM
Vlp15/16 B.miyamotoi	OspC B.afzelii	P58 B.afzelii	ABM	ABM	ABM	ABM	ABM	ABM	ABM
Vlp18 B.miyamotoi	P17 B.garinii	IgG, 10uf/ml	ABM	ABM	ABM	ABM	ABM	ABM	ABM
buffer	P17 B.afzelii	IgM, 10ug/ml	ABM	ABM	ABM	ABM	ABM	ABM	ABM

Clinical samples

Patients in which blood sample, taken at admission, B.miyamotoi 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.miyamotoi 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)

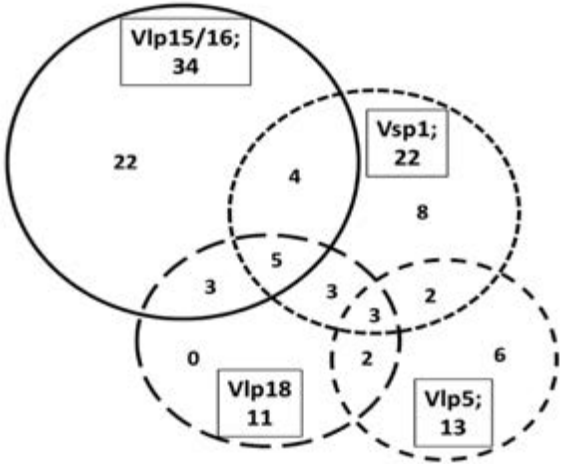
4 – day 340-365 after symptoms onset (n =5)

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

Protein	GlpQ	Vsp1	Vlp5	Vlp15/16	Vlp18	B.b.s.l.
Group 1						
IgM pos/total	19/24	12/24	10/24	15/24	4/27	19/24
IgM appearance	Day 6	Day 6	Day 7	Day 6	Day 49	Day 7
Group 2						
IgM pos/total	23/26	6/26	11/26	13/26	3/26	13/26
IgM appearance	Day 9	Day 9	Day 7	Day 7	Day 7	Day 7

Protein	GlpQ	Vsp1	Vlp5	Vlp15/16	Vlp18	B.b.s.l.
Group 1						
IgG pos/total	21/24	4/24	13/24	18/24	2/24	3/24
IgG appearance	73 day	73 day	63 day	79 day	20 day	73 day
Group 2						
IgG pos/total	16/26	7/26	10/26	14/26	2/26	4/26
IgG appearance	29 day	30 day	10 day	16 day	30 day	25 day



Visual presentation of the spectrum of responders to the different Vmps among 50 patients with PCR confirmed *B. miyamotoi* disease

Numbers in the outer part of ovals are the numbers of patients only responding to the indicated Vmp (i.e. Vlp-15/16, Vlp-18, Vsp-1). Numbers in the intersecting part of ovals are the numbers of relevant double- or triple-responders.
A patient was marked as a responder to an antigen when either IgM or IgG antibodies directed against this antigen were above the threshold in any of the replicate sera.

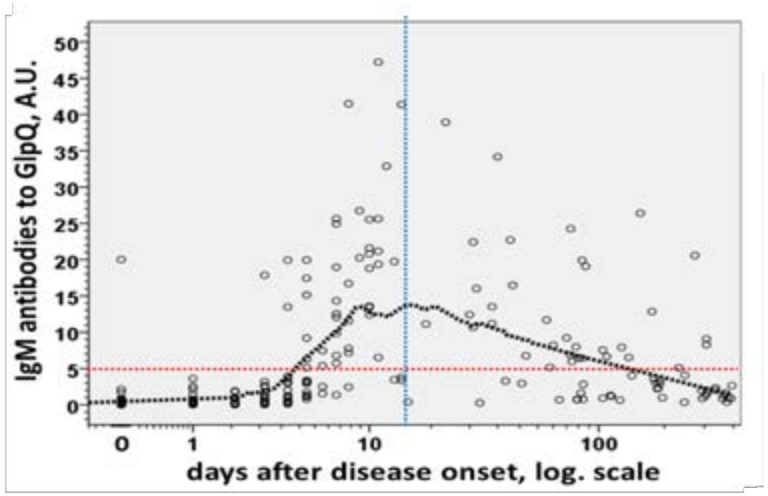
Seroconversion of antibodies specific to antigens of B. miyamotoi and B. burgdorferi s.l. in blood serum samples of patients during hospitalization

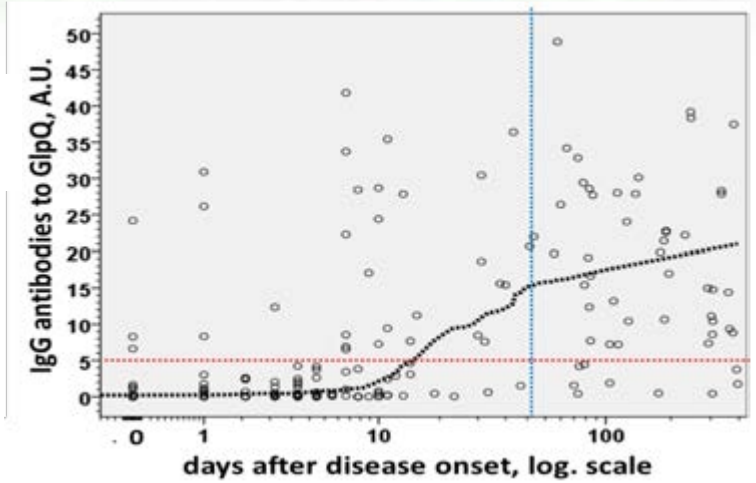
	Group 1		Group 2	
Day after symptoms onset	IgM	IgG	IgM	IgG
1-3	0/24	0/24	0/26	0/26
6-9	16/24	4/24	18/26	9/26
10-14	4/24	2/24	5/26	2/26
∑, 6-14 day	20/24	6/24	23/26	11/26
∑, seroconversion	21/24		23/26	

* The average duration of hospitalization for a disease etiologically associated with B. miyamotoi is 10 days.

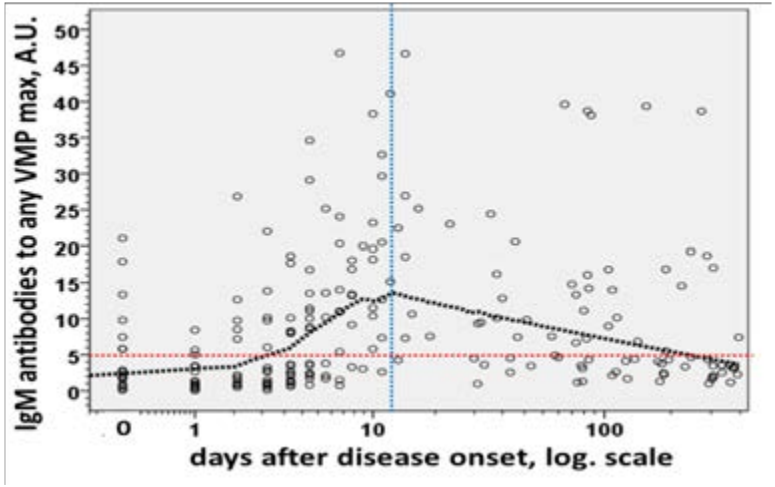
Detection of GlpQ-specific antibodies in blood serum samples of patients for the entire follow-up period (n=222)

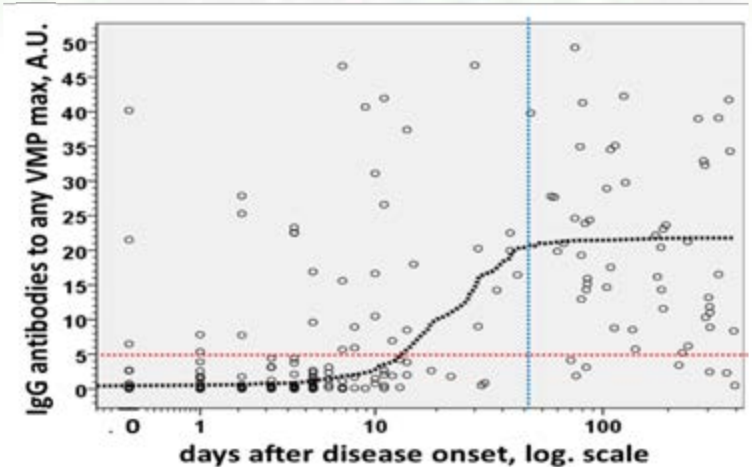
One arbitrary unit (AU) corresponds to 1 µg/ml of IgM or IgG antibody





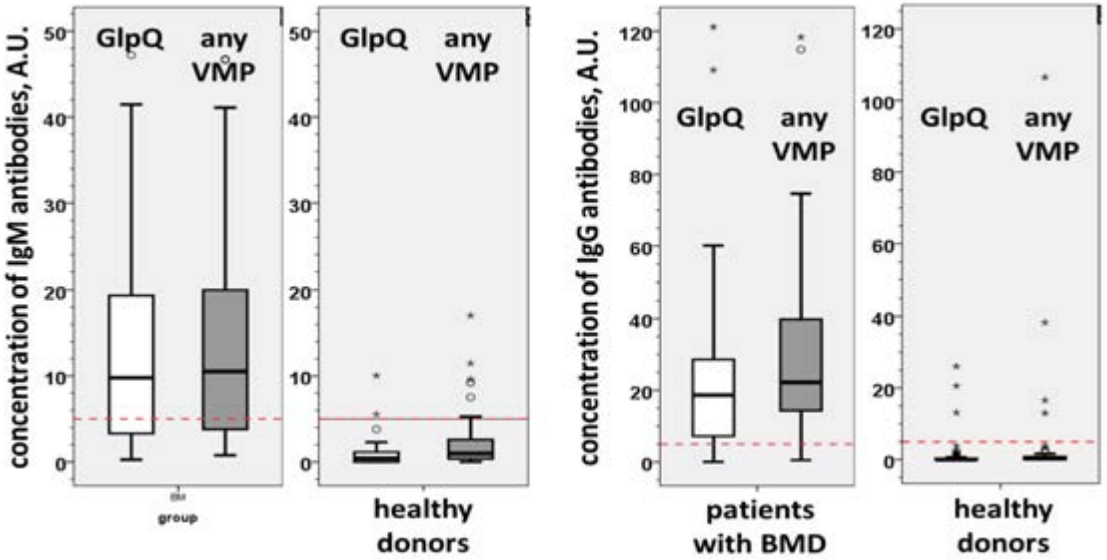
Detection of VMPs-specific antibodies in blood serum samples of patients for the entire follow-up period (n=222)





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

		Group 1	Group 2	control
IgM	GlpQ	20/27	23/26	1/61
	Two Vlp/Vsp without Ab to GlpQ	3/27	1/26	-
	B.b.s.l. without Ab to GlpQ & Vlp/Vsp	1/27	1/26	1/61
IgG	GlpQ	23/27	16/26	4/61
	Two Vlp/Vsp without Ab to GlpQ	2/27	1/27	-
	B.b.s.l. without Ab to GlpQ & Vlp/Vsp	-	-	2/61
IgM&IgG to GlpQ/two Vlp/Vsp		21/27	18/26	-
IgM/IgG to GlpQ/twoVlp/Vsp		26/27	24/26	5/61
IgM/IgG to GlpQ/two Vlp/Vsp/ B.b.s.l.		27/27	25/26	8/61



The concentration of protein-specific antibodies to B.miyamotoi antigens, in healthy donor groups (n=70) and 50 patients with ICD, determined by B. miyamotoi.

Any VMPs is the highest value of the 4 VMP used for a particular sample.

Data for patients with B.miyamotoi-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 pireod (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 ones 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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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.