DYNAMICS OF ANTIBODIES IN *BORRELIA BURGDORFERI SENSU LATO* INFECTIONS

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DYNAMIKA TVORBY PROTILÁTOK PRI INFEKCIÁCH *BORRELIA BURGDORFERI SENSU LATO*

Abstract

Kmety E:
Dynamics of antibodies in *Borrelia burgdorferi sensu lato* infections
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Antibody response in infections with *Borrelia burgdorferi sensu lato* is generally considered to be slow and delayed, but exact studies concerning this question are hardly found in the scientific literature.
During 1994—1998 at least two serum samples were submitted for serological testing from more than 1200 patients. An immunofluorescence test was performed paralelly with two pools of antigen (*B. bg.s.s.* + *B. afzelii*, and two serological different strains of *B. garinii*, all of local origin).
In 92—96 % of patients no change of antibody level was found in repeated tests, about 20 % of them being negative (<1:512). In 2—4 % of cases a significant increase and in less than 1 % a decrease or a second wave of the rise of the antibody level was observed. From the 58 patients with increasing antibody levels 9 had ECM (mostly children), 2 cardiovascular, 8 musculo-skeletal and 12 neurological disorders. In the remainig cases the diagnosis was not specified (A 69).
Only in 9 cases a rise of the titer appeared during 3 weeks after the first negative sample,at contrary in 7 cases no rise of the titer was seen in that time. 2 patients were still after 1 month, 3 after 3 months and 1 even after 7 months (patient with a positive CSF culture) serologically negative.
A 2 titer step decrease was observed in 4 cases during 1 year and once a 3 titer decrease after 4 years.
Concl*usion:* A rise of antibodies can be seen in all clinical manifestations of Lyme disease with prevalence in the early types of the disease. The rise of antibodies is often delayed or even inhibited, apparently mostly by an early efficient treatment. Whether a late rise of antibodies or its long persistence indicates a latency of the infection, requires more detailed studies. *(Tab. 5, Ref. 6.)*
Key words: Lyme borreliosis, dynamics of antibodies.

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Abstrakt

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Dynamika tvorby protilátok pri infekciách *Borrelia burgdorferi sensu lato*
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Tvorba protilátok sa pri infekciách *Borrelia burgdorferi* všeobecne považuje za pomalú a oneskorenú, exaktné štúdie k tejto otázke sú však v odbornej literatúre veľmi skromné.
Počas rokov 1994—1998 sme vyšetrili aspoň 2 vzorky séra v priebehu ochorenia od viac ako 1200 pacientov sérologicky nepriamym imunofluorescenčným testom za použitia 2 zmesí antigénov (*B. bg.s.s.* + *B. afzelii* a dva sérologicky odlišné kmene *B. garinii*, okrem kmeňa B 31 všetko endemické kmene).
U 92—96 % pacientov nedošlo v ďalších vzorkách séra ku zmene titra (asi 20 % vzoriek bolo opakovane negatívnych (<1:512)), u 2—4 % výrazne stúpli a u menej ako 1 % klesli alebo v druhej vlne stúpli hladiny protilátok.
Z 58 pacientov, u ktorých sme zistili výrazný vzostup protilátok, mali 9 diagnózu ECM (väčšinou deti), 2 mali kardiovaskulárne, 8 muskulo-skeletálne a 12 neurologické poruchy. U ostatných nebola diagnóza bližšie špecifikovaná (A 69).
Iba u 9 pacientov sme vzostup protilátok zaznamenali do 3 týždňov od prvej negatívnej vzorky, 7 nedávali v tomto čase pozitívnu reakciu, 2 však ani po 1 mesiaci, 3 ani po 3 mesiacoch, 1 ani po 4 mesiacoch a 1 ani po 7 mesiacoch (pacient s pozitívnou kultúrou z likvoru).
Pokles titru o 2 stupne sme pozorovali v 4 prípadoch v priebehu roka a raz o 3 stupne po 4 rokoch.
Závery: Signifikantný vzostup protilátok možno pozorovať pri všetkých klinických manifestáciách lymskej boreliózy, predovšetkým však pri jej skorej forme. Býva často oneskorený alebo viac-menej inhibovaný, pravdepodobne v dôsledku skorej účinnej terapie. Otázka, či dlhodobá perzistencia protilátok indikuje určitú latenciu infekcie, však vyžaduje osobitné štúdium. *(Tab. 5, lit. 6.)*
Kľúčové slová: lymská borelióza, dynamika tvorby protilátok.

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Antibody response in infections with Borrelia burgdorferi sensu lato (*B. bg.s.l.*) is generally considered to be slow and delayed (Johnson, 1997), but exact studies devoted to this question are hardly found in the scientific literature.

In our study an attempt was made to approach this problem by evaluating serological results in more than 1200 patients being at least twice submitted to serological tests.

Material and methods

During the last five years at least two serum samples taken in different time intervals were obtained from 1215 patients. The samples were serologically tested by the indirect immunofluorescence test (IIFT) using paralelly two antigens, in the first years the standard strain *B. bg.s.s.* B 31 and our endemic strain of *B. garinii* K 48 (OspA serotype 6) (Kmety, 1990; Wilske, 1997) later the B 31 antigen was pooled with an endemic strain of *B. bg.s.s.* and *B. afzelii,* and the strain K 48 with an other endemic antigen of *B. garinii* (K 24 or K 1, OspA serotypes 3, resp. 5) (Mateička, 1998).

If titer differences between the two antigen pools appeared (only exceptionally more than (one titer), the lower titer was taken into consideration. The cut off level was 1:512. An increase of at least 2 titer steps in comparison with the higher reacting pool of antigens was considered to indicate a significant rise of the antibody level.

Because responsible data of the beginning of the illness were in the great majority of cases hardly available, the succeeding serological results were compared with the result of the first sample, which might have been taken, at least in the majority of cases, in the first weeks of the illness.

Besides the greatest part of patients comming from a neurological or dermatological clinic a growing number were ambulatory outpatients patients of practitioners. Therefore the exact diagnosis was in most cases not sufficiently specified. A few samples were submitted also from healthy persons after tick bites.

Tab. 1. Number of patients with succeeding serological testings.
Tab. 1. Počet pacientov s opakovaným sérologickým testovaním.

Year	n	Repeatedly posit.	Repeatedly negat.	Titer increase	Titer decrease	Titer down-up	data incompl.
1994	429*	261	133	29	1	1	4
1995	220	161	45	9	1	–	4
1996	217	138	64	11	1	1	2
1997	171	112	51	5	1	1	1
1998	180	148	27	4 (2.2%)	1	–	-
	1217	820 (93,6%)	319	58 (4,8 %)	5 (0,4 %)	3	11

* including patients having the first sample tested in 1993

Tab. 2. Diagnosis of patients with increasing antibody levels.
Tab. 2. Diagnóza pacientov so zvýšenými hladinami protilátok.

1.	Erythema migrans	–	9	(m – 1,f – 8)	– average age 23 y.
2.	Neurological sy.	–	8	(m – 2,f – 6)	– " " 35 y.
3.	Musculo-skelet.sy.	–	6	(m – 2,f – 4)	– " " 25 y.
4.	Cardiovascular sy.	–	2	(m – 1,f – 1)	
5.	Ocular sy.	–	1	(m – 0,f – 1)	
6.	healthy persons (tick bite)	–	3	(m – 2,f – 1)	
7.	LB or A 69	–	24	(m – 9, f -15)	
8.	others	–	5	(m – 3, f – 2)	
			58	(m – 20, f – 38)	

Results

From the 1217 patients 1139 (93,6 %) showed in succeeding samples no change in their titer levels, about 2/3 of them being repeatedly high titer positive and somewhat less than 1/3 of them repeatedly negative (<1:512 with both pools of antigens).

In 58 patients (4,8 %) a significant increase of the titer in comparison with the first sample, was observed. A decrease of the titer was found in 5 patients (0,4 %) and in 3 patients after an inicial decrease a new wave of rising antibodies appeared (Tab. 1).

Concerning the group of patients with rising antibody titers it was interesting to see that 2/3 of them were females. Only in 9 cases the diagnosis *Erythema migrans* (EM) was reported, mostly in children, but a number of them may be hidden under the unspecified diagnosis of Lyme borreliosis (LB).

From the practical point of view it should be stressed, that a rise of the titer was observed also in patients with neurological, cardiovascular or musculo-skeletal disorders. These observations are based only in 20 out of 58 cases on the results of two samples only (Tab. 2).

The increase of the titer was observed only in 8 cases within 2 weeks after the first negative sample, during 3 weeks the number of positive patients increased to 11. In the remaining 47 cases the rise of the titer was firstly detected after 1—3 months and later. Because this depends in reality on the time the samples were submitted for investigation, more imporant is the time, at which the second or third samples were still negative (Tab. 3).

15 patients out of the 58 with increasing antibody levels had one or more negative serological tests after weeks, but even after months, before the appearence of an high antibody titer in their serum sample. In one case of retrobulbar neuritis (proved by a positive CSF culture),even after 7 months no significant rise of the titer was found.

In five cases a decrease of the titer by 2—3 steps was observed. The reasons of this remains unclear.

Interesting were 3 cases showing a significant decrease of the antibody titer within about one year, but apparently a relaps of the infection or a new infection caused that the titer rised again.

Discussion

Doubts about the usefulness of serological tests in LB are not seldom. Justified is therefore the question, what can we exac-

Tab. 3. Number of patients repeatedly negative before the increase of the antibody level.
Tab. 3. Počet opakovane negatívnych pacientov pred nárastom hladiny protilátok.

1	patient	2	weeks	(after the first
6	"	3	"	negative sample)
3	"	1	month	"
2	(+ 2)*	2	"	"
1	(+ 1)*	3	"	"
1		4	"	"
1		7	"	(CSF culture +)
15	(+ 3) – *	2	serum samples negative after the first negative one	

tly expect from these tests to support or correct the clinical diagnosis.

Our study was based on results of the IIFT using paralelly pools of two antigens prepared from endemic strains as recommended by EUCALB (European concerted action on Lyme borreliosis) (Guy et al., 1998). The high consistency of serological results found in over 93 % of patients with repeatedly positive or negative serum samples, seems the IIFT to provide a reliable basis for our comparative study.

The rise of the antibody titer — in many infectious disease a convincing diagnostic marker, is in LB expected to be seldom observed because of a delayed antibody production and if, only in its early clinical manifestations. From our studies follows that a significant increase of the antibody titer may be observed not only in cases of EM or other types of early LB, but also in other clinical manifestations of this illness, mainly neurological, cardiovascular, musculo-skeletal or ocular, however more seldom.

The considerably delayed appearence of antibodies in cases of fresh LB indicates the necessity to repeat the testings in longer time intervals. A number of patients were even after few months after the first negative sample serologically negative.

A particular attention deserves a patient with retrobulbar neuritis diagnosed by a positive CSF culture (*B. afzelii*), who was even after 7 months after the onset of the illness serologically negative, probably because of an initial treatment with corticoids, and after one months, when the diagnosis was established, with high doses of antibiotics. Only after one year a significant rise of the antibody titer was finally found.

In patients remaining under clinical control for years, no decrease of the antibody titer was seen (with one exception).

From these observations follows that even after repeated negative serological results obtained in shorter time intervals the diagnosis LB cannot be definitively excluded, especially not after antibiotic therapy. In a number of cases our clinicians were surprised that only a control test after more months proved their presumed clinical diagnosis.

It is evident that during the years the number of patients with increasing levels of antibodies considerably decreased, an observation which may indicate a better knowledge and more experiences in diagnosis and adequate therapy, accomplished in the last years.

In a few patients a decrease of antibodies was obeserved, in 3 cases by 3 titers after 4 and 8 months resp., once after 4 years and in 2 cases by only 2 titer steps.

Interesting was the observation in a few cases, where after an initial decrease a new wave of rising antibodies was detected. Insofar as it was accompanied by a compatible clinical symptomatology, it may be interpreted as a relaps of the illness caused probably by ineffective therapy. However the possibility of a new infection cannot be excluded.

Not seldom appears the question whether the persistence of high titer antibodies is indicating a latency of the infection or just a postinfectious residuum. This question requires additional scientific efforts and evidently also other methods (PCR?).

Conclusions

The appearence of borrelial antibodies, mainly in patients treated with antibiotics, is usually delayed for weeks and even months. Consequently repeatedly seen negative serological test within short time intervals do not necessarily exclude borrelial infection.

Tab. 4. Patients with decreasing antibody levels.
Tab. 4. Pacienti s klesajúcimi hladinami protilátok.

after	4	months	–	2	titer	steps
“	8	“	–	2	“	“
“	10	“	-	3	“	“
“	1	year	–	2	“	“
“	4	“	–	3	“	“

Tab. 5. Patients with increasing antibody levels — time after the first negative sample.
Tab. 5. Pacienti so stúpajúcimi hladinami protilátok — v čase po prvom negatívnom výsledku.

Time	Number of patients	Number of tested succeeding samples
< 2 weeks	8	7 x 2, 1 x 7,
3 weeks	3	2 x 2, 1 x 5,
1 month	10	3 x 2, 3 x 3, 3 x 4, 1 x 6,
2 months	5	2 x 2, 1 x 3, 1 x 4, 1 x 5,
3 months	10	2 x 2, 3 x 3, 2 x 4, 1 x 5, 1 x 7,
4 months	7	3 x 3, 3 x 4, 1 x 7,
5 months and more	15	2 x 2, 6 x 3, 3 x 4, 1 x 5, 1 x 6, 1 x 8, 1 x 9.

A rise of the antibody level may be seen not only in patients with early types of LB, but also, however more seldom, in cases with neurological, cardiovascular, musculo-skeletal or ocular lesions.

Whether the long persistence of high titer antibodies is indicating a borrelial infection in a state of latency or not, requires more detailed studies.

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