We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

3

Advancement in *Borrelia burgdorferi* Antibody Testing: Comparative Immunoblot Assay (COMPASS)

András Lakos[1] and Erzsébet Igari[2]
[1]*Centre for Tick-borne Diseases, Outpatient Service and Laboratory*
[2]*Semmelweis University, Faculty of Medicine*
Hungary

1. Introduction

Since the discovery of the Borrelia burgdorferi s.l. (Bb) in 1982 (Burgdorfer et al., 1982), we can test antibodies against it. We could establish the diagnosis of Lyme borreliosis (Lb) and heal many-many patients by the help of this technique. Soon after the discovery of the pathogen, it became apparent that the serological tests are not sufficiently specific (Banyas, 1992; Cutler et al. 1994; Tuuminen et al., 2011). Generally, cross reacting antibodies (like in syphilitic patients) are accused for this type of bias (Craft et al., 1984; Raoult et al., 1989; Rath et al., 1994), but it is questionable whether most of the false positive reactions are originated from these cross reactive antibodies. The only diagnostic tool for supporting Lb in a routine laboratory is testing antibodies against Bb. Recently, the relatively cheap ELISA is most frequently used. This technique generally measures the amount of antibodies against different Bb antigens, and it is quantitative. Since ELISA detects more than one antibody we can not reveal which antibody gives the "positive" result. In an ELISA test the background noise and non specific reactions may be significant therefore it is generally accepted that test results provided by ELISA should be confirmed by Western (immuno) blot (Wb), this called as "two-step protocol" (CDC, 1995). Wb measures the different, most and less specific antibodies separately therefore it is considered to be more specific, but expensive method. The drawback of the Wb is that it is although qualitative but only semi quantitative. Most of the guidelines do not consider calculation with the band intensity at all (CDC, 1995; Dressler et al, 1993; Hauser et al, 1997). This mistake can be avoided by applying computer image analysis of Wb where intensity is also measured in comparison to factory given controls (Binnicker et al; 2008).

Problem 1/ In spite of that significant development is seen in methodology and test performance (Bacon et al., 2003; Porwancher et al., 2011; Steere et al., 2008), the test quality can be seriously different (Ang et al, 2011, Lakos, 1990; Marangoni et al., 2005; Nohlmans et al., 1994,). At least a part of the false positive results inevitably originate from a previous infection. The highest specificity ever published from an endemic population was 99.1%. (Goettner et al., 2005). Most of the tests have much lower specificity. Even the 99% specificity is excellent, we found that the positive predictive value (PPV) of this test is surprisingly low (less than 10%) when it is applied as a screening test in an average population living in endemic region (Lakos et al., 2010).

Since Bb antibody testing will result in at least 1% false positive result in endemic territories (practically in entire Europe), and basically, this false positivity is the consequence of a previous infection, this bias probably cannot be unravelled by newly developed serological techniques.

Problem 2/ The situation is even worth when Bb antibody testing is applied in people (forestry workers, hunters, orienteer, mushroom pickers and bee-keepers) with high risk for Lb. We found that among 1670 forestry workers 622 (37%) were seropositive and 280 (45%) of them was free of any symptoms and they also denied any symptom suggestive of Lb in their history. Therefore the PPV in this population is even lower, 1.8% (Lakos & Igari, 2011).

Problem 3/ Most of the guidelines follow the case definition of EM published by CDC (CDC, 2008) and EUCALB (Stanek et al., 1996). But there is no instruction what a clinician should do in a case when the rash does not fulfil the diagnostic criteria of EM but otherwise Bb infection can not be ruled out safely. In such cases, clinicians order antibiotic treatment for sure what is sure. Delusion is very probable in such cases and the consequences are regularly serious (Steere et al., 2004). How can we prove that the patient does not need antibiotic treatment?

Problem 4/ It is a basic rule that a symptom free patient must not be treated with antibiotics just because of a ”positive” serological result. A competent physician neither treats a symptom free patient with a previous symptom suggestive of Lb. How can one sure that being free of symptoms means being free of infection?

Problem 5/ Facial palsy is a frequent complication of Lb. Tick bite usually resides behind the ear in these cases, therefore rarely recognised. EM is usually missing, therefore the only diagnostic tool is Bb antibody testing. Since facial palsy is an early complication, the antibody reaction is still negative in many cases. Situation is complicated by steroid treatment what is usually applied based on studies showing facial palsy improved by corticosteroids (Numthavaj et al., 2011; Sullivan et al., 2007). This treatment may modify antibody reaction and help propagation of Bb. The Lyme spirochete frequently invades the central nervous system but in spite of that it does not result in apparent clinical symptoms (Ackermann et al., 1984; Pachner & Steere, 1984). Facial palsy caused by Bb infection is usually benign, self limited illness. These increase the chance of being hidden the origin of the disease. What should a doctor do when the serology is negative shortly after the debut of facial palsy?

Problem 6/ Studies have been published supporting that Bb is able to survive the adequate antibiotic treatment (Kannian et al., 2007; Maraspin et al., 2002; Steere & Angelis, 2006; Wormser et al., 2003). To prove this in a given patient, biopsies and culture or PCR should be applied but sensitivity of these techniques after treatment is questionable. These methods are expensive, usually invasive and time consuming; therefore they are inappropriate for routine diagnostics. What about serology? Is it the right way for demonstration of Bb survival?

Problem 7/ It is generally accepted that demonstration of intrathecal antibody production is necessary for supporting neuroborreliosis. The basic problem is that leakage of the blood-brain barrier may result in appearance of the peripherally produced immunoglobulin in the cerebrospinal fluid (CSF). We should differentiate between the intrathecally and peripherally produced antibodies presented in the CSF. Some techniques have been developed for solve this problem. Most of these apply four measurements (e.g. serum IgG anti-Bb antibodies / total serum IgG antibodies and CSF IgG anti-Bb antibodies / total CSF IgG antibodies).

Mathematical equations were developed as well as arbitrarily defined factors were applied and the usefulness of these was supported by empirical data (Halperin et al., 1991; Kaiser, 2000; Wilske et al., 1986). The more measurements we take, the more mistakes we can make. The most serious mistakes may present in cases where the amounts of immunoglobulins are excessively low or high as just this situation is typical for Bb infected patient without neuroborreliosis and the non-Lyme patients. What possibilities we have to achieve safer results with fewer measurements?

Problem 8/ A lot of papers show that about 10% of the adequately treated patients develop subjective symptoms (fatigue, cognitive impairment and musculoskeletal pain) months or years later. These complaints named "post Lyme syndrome" can not be improved by antibiotic treatments (Klempner et al., 2001, Marques, 2008; Wormser et al, 2006). But we can not rule out that these symptoms are resulted from surviving Bb. Ineffectiveness of antibiotic treatment(s) is not enough for proving that post Lyme syndrome is not a consequence of long lasting Bb infection. Could we find more support to clarify this?

Problem 9/ A lot of papers on the effectiveness of antibiotic treatment in Lb have been published but exaggerated guidelines appeared. For example, 10 days (Wormser et al., 2003) versus 30 days (Stanek & Strle, 2003) doxycyclin treatment were also suggested. Moreover we found a study applying 11 months long treatment (Donta, 1997). In contrast, azithromycin described to be effective in a five day course (Arnez et al., 2002, Hunfeld et al., 2005). The situation is more complicated since the treatments are suggested to be shorter in the early forms than in the later. What is the reason of the difference? Is there any evidence for this practice? More recently, neuroborreliosis is suggested to be treated by doxycycline with a similar dose as we use it in the treatment of EM. With this treatment we may not achieve MIC or MBC levels of some Bb isolations in the CSF (Baradaran-Dilmaghani & Stanek, 1996; Karlsson et al., 1996; Kleibeuker et al., 2009). Treatment of pregnant women is also a special issue. The guideline of IDSA (Infectious Disease Society of America) claims that pregnant women should be treated by the same dose of amoxicillin as other Lyme patients (3x500mg/die) (Wormser et al., 2006). In turn of this statement the metabolism of amoxicillin is faster in pregnancy, moreover the increased body weight and relatively higher amount of body fluid result in lower antibiotic level than needed (Andrew et al., 2007). We found that orally treated pregnant women has almost the same chance for adverse outcome as the untreated women in contrast with the treatment with ceftriaxone, that resulted in almost 100% safe in preventing the complications (Lakos & Solymosi, 2010). Lb is usually benign and self limited disease, therefore judgement of the effectiveness of an antibiotic treatment would need a study on a rather big population. Clinical improvement does not imply per se that microbiological cure was also achieved. On the contrary, if the patient complains for persistent or relapsed symptoms it does not necessarily mean that Bb survived the antibiotic treatment. Designing of a double blind study for the comparative effectiveness of an antibiotic treatment is quite complicated. Since the photosensitive reaction of doxycycline we easily recognise which treatment was applied before the patient would sound. The nasal redness is very characteristic sign of doxycycline treatment during the summer (i.e. Lyme) season and rarely missing. The double blind setting is definitely hurt when ceftriaxone treatment is tested since the characteristic smell of the antibiotic discloses the real drug for the patient and physician as well. Therefore a laboratory method would be better to test the effectiveness of the antibiotic instead of analysis of the subjective data of symptoms.

We developed the comparative immunoblot assay (COMPASS) to solve the above problems.

2. Methods

2.1 Western (immuno) blot

Bb (strain ACA1) was disintegrated by ultrasound than proteins were separated by SDS polyacrylamide gel ELFO. These proteins were transferred (blotted) to a polyvinyl difluorid membrane. This membrane is more durable than the gel, and the immunological reaction is performed on this membrane, that is the first layer. We put on the diluted serum or CSF sample to be tested. The antibodies strongly connected to the antigens therefore the repeated washing can eliminate all the serum components except the bound antibodies. The next step is the application of an anti-human immunoglobulin which was produced by an animal. This was conjugated by the producer factory with an enzyme which can develop the colouring reagent that is insoluble and remains in the membrane, showing the location of the bound antibodies (bands) as well the intensity of the binding. This intensity reflects to the concentration of bound antibodies. We waited for the development of the appropriate intensity than we apply the stop reagent, the last step of the serological reaction. The band intensity is merely influenced by the quality and concentrations of each layer. We establish the optimal concentration of each layer by checker board dilution. The location of a given antigen/antibody was originally defined with the help of monoclonal antibodies. Than we selected positive control samples for IgG and IgM and signed the appropriate molecular weights to a scanned image of these positive control samples. With the help of this scanned and signed image we can easily locate the bands during the routine examinations. We subjectively judge the intensity of the bands comparing the control samples. The positivity/negativity (i.e. cut-off) of a given Wb test can be defined by different ways. We tested 300 clinically defined Lyme patients (EM, lymphocytic meningoradiculitis and acrodermatitis chronica atrophicans - ACA) and 300 controls (healthy blood donors, infants and patients with autoimmune diseases). Based on these studies, the most specific bands were: 23, 29, 35, 44, 47, 49, 93 kDa molecular weights in IgG; 23 and 41 kDa in IgM. We analysed also the intensity of the bands (Lakos & Granström, 1997).

2.2 COMPASS

This method was described earlier in some respect. Accordingly, in most cases, two samples were drawn with an interval. The first sample was stored in a deep freezer and then tested together with the second sample when it was drawn later. These samples are tested side by side in Wb, therefore the difference or sameness can safely be judged, and the technical bias is practically avoided. These are partly described in previous papers (Lakos et al., 1997, 2005, 2008). We evaluate the difference between the samples and not the "positivity/negativity". Progression means persistent, lack of progression represents past infection. There are specific applications of COMPASS when the two samples drawn on the same day i.e. testing intrathecal antibody production, when serum and CSF samples are compared or testing intrauterine infection when serum samples of the mother and the newborn are compared. Sample pairs are always tested side by side.

2.3 Patients

There were some patients who remained untreated; their data are extremely useful for improving our knowledge on that how Bb antibodies develop in time. Patients remained untreated from different reasons: some of them refused the antibiotic treatment and used

alternative medicine, e.g. homeopathy. In some other, the first sign of EM was not typical or smaller than 5cm in diameter but later the clinical appearance turned to be definitive. There were patients who had no symptoms at the first visit but because of multiple tick bite we tested a serum sample with negative result. Weeks later EM developed and the second sample was drawn.

2.4 Ethics

This study was approved by the Scientific and Ethical Committee of Medical Research Council (2409-0/2011-EKU - 66PI/11), based on the review of the detailed description (objective, background, hypothesis, study design - collecting patients, their clinical and laboratory data, the number and age of the participant patients). All patients provided a written informed consent: "I give my permission to Dr. Lakos to use the clinical and laboratory data collected on my (or my child's) illness for scientific analysis without mentioning my name and other personal information. This personal information must not be shared with other person. I am aware that have the right to withdraw my permission before the study will be closed." The study complied with the principles laid down in the Declaration of Helsinki.

3. Examples

In this section we illustrate the usefulness of COMPASS in different situations, and present some cases to show the process of antibody response to Bb infection in untreated patients.

3.1 COMPASS for serological confirmation of microbiological cure

3.1.1 Example 1

A, 26.09.1996. The 80 years old woman showed typical clinical signs of ACA. The first serum sample was drawn 18 months after the first treatment administered at another institute. She received IV penicillin for 8 days than 1x100mg doxycycline orally for 7 days. The size of the inflamed region decreased but the pain remained at the region of ACA. Concerning the extreme positive antibody response and complains we prescribed doxycycline and azithromycin. A year later arthralgia and polyneuropathy developed; therefore we ordered antibiotic treatments again.

B, 24.02.2000. By this time she received 5 courses of antibiotics altogether for 137 days, among these ceftriaxone was administered in twice regimens.

C, 21.05.2002. This is the last sample, almost 6 years after the first. COMPASS shows the definitive serological regression in spite of that the last sample is still strongly positive. Most of the bands faded, antibody against OspC disappeared (arrows).

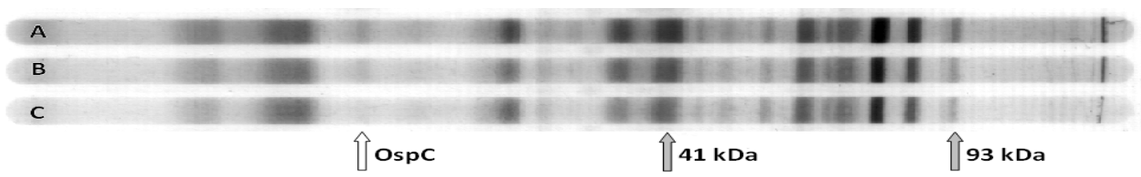


Fig. 1. Serological follow up in a patient with ACA (IgG)

3.1.2 Example 2

A, 23.08.2010. Patient was treated with amoxicillin for EM.

B, 18.06.2011. The intensity remained unchanged in all but one bands. In spite of the result is still positive, microbiological cure is proven.

Fig. 2. Clinical and serological recovery (IgG)

3.1.3 Example 3

A, 16.08.2010. EM located behind the knee and accompanied by serious pain in the leg. He was treated with doxycycline for 40 days 100mg b.i.d.

B, 18.06.2011. He is free of any symptoms. Although the result is still positive but there is no progression, and the antibody against the 44kDa protein decreased, therefore microbiological cure is proven.

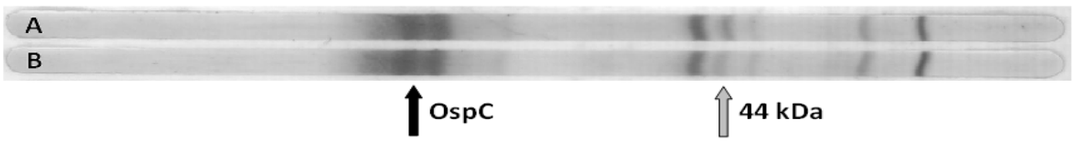


Fig. 3. Another example for clinical and serological recovery (IgG)

3.1.4 Example 4

She had a typical EM from May to October 2009, and visited our Centre shortly after the abortion of her 8 weeks old pregnancy. The EM was still high coloured, and disappeared during amoxicillin treatment.

A, 22.07.2010. This sample was drawn nine months after the treatment for proving microbiological cure.

B, 02.02.2011. She asked for serology because of arthralgia. Since no definitive progression was seen, she was not retreated.

C, 15.06.2011. The woman asked for this test before planning a new pregnancy. A definitive decrease is seen in the reaction against the 14kDa protein. The minimal progression in the 41kDa (flagellar) antibody turned to be temporary. This is a good example for that serological regression may be seen long after the treatment.

Fig. 4. Another example for clinical and serological recovery (IgG)

3.1.5 Example 5

Tetraplegia developed gradually with chronic lymphocytic meningitis in April 1994.

He was treated with ceftriaxone in February 1995, than with doxycycline in November 1997. He improved a lot, able to walk without any support but clumsiness in coordination progressed since a year.

A, 01.12.1997.

B, 21.03.2005.

C, 09.06.2011. This is a good example for that many years after healing of a serious, long lasting Bb infection the serological result is still strongly positive. Proving the micro-biological cure would not be possible without COMPASS which shows moderate decrease in antibody response.

Fig. 5. Healing of progressive Borrelia encephalomyelitis (IgG)

3.2 COMPASS suggesting Borrelia survival

3.2.1 Example 1

A, 11.08.2010. EM started 25 days before sampling. The IgM serology was strongly positive. She was treated with amoxicillin 1000mg t.i.d.

B, 08.12.2010. The next sample showed serological progression but she was not retreated.

C, 15.06.2011. In the third sample a minimal further progression is shown (striped arrow). Another antibiotic treatment was prescribed.

Fig. 6. Serological progression after clinically successful treatment of EM (IgG)

3.2.2 Example 2

Arthritis of the right knee developed in April, 2010. Other large joints became intermittently swollen.

A, 05.04.2011. He was treated with 100mg doxycycline b.i.d., but the arthritis persisted. Ceftriaxone was ordered 1x2g IV for 15 days. The inflammation seemed to be healed but one week after finishing the treatment left knee became swollen again (17.06.2011.).

B, 23.06.2011. Almost every band shows progression (striped arrows) but some new bands also appeared (dotted arrows). We have not seen similar serological progression after successful treatment.

This example also represents that the very strong reaction can progress to a more reactive form. Lyme arthritis always accompanied by similarly strong antibody reaction as in acrodermatitis chronica atrophicans and progressive encephalomyelitis.

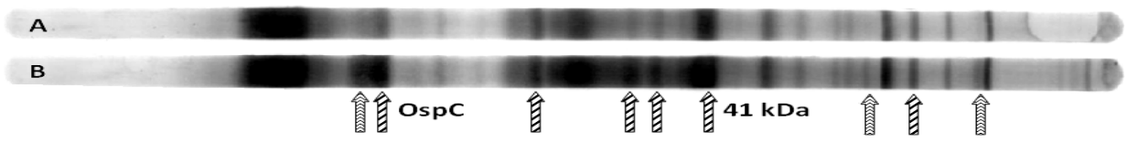


Fig. 7. Seroprogression after the ineffective treatment of Lyme arthritis (IgG)

3.2.3 Example 3

A, 09.08.2010. EM started 4 days before sampling. He was treated with amoxicillin for 20 days.

B, 02.12.2010. Minimal progression was seen in OspC (striped arrow), but he was not retreated at this time.

C, 22.06.2011. Further progression is seen in 44kDa antibody. Treatment was ordered.

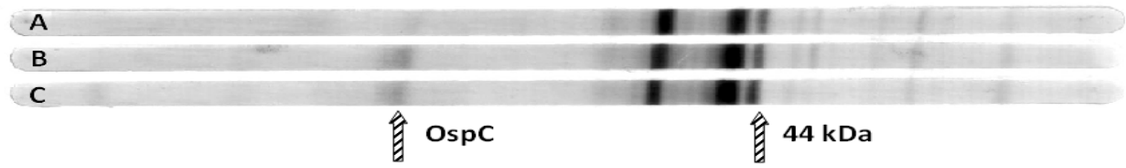


Fig. 8. Seroprogression after clinically successful treatment (IgG)

3.2.4 Example 4

A, 30.09.2010. A tick was recognised behind the ear one month before. Peripheral facial palsy started three days before sampling. Strong reaction was seen in IgM. Facial palsy was healed one week after starting the ceftriaxone therapy (2g IV, per day).

B, 09.12.2010. Definitive progression was seen in IgG but antibiotic treatment was not ordered at this time.

C, 15.06.2011. Further progression is seen, antibiotic treatment was prescribed.

Fig. 9. Serological progression after clinically successful treatment (IgG)

3.2.5 Example 5

A, 30.08.2010. She was treated with two courses (17 days in total) of azithromycin because of peripheral facial palsy with strong positive Bb antibody IgM reaction. She visited our Centre one week after healing of the palsy and just after finishing the treatment.

B, 30.11.2010. Serological regression was visible in the 44kDa band.

C, 31.05.2011. Progression appeared (arrow) half year later in the same band. We assessed this phenomenon - ie. the transitory serological regression followed by progression - as a sign of Bb survival. The patient was retreated.

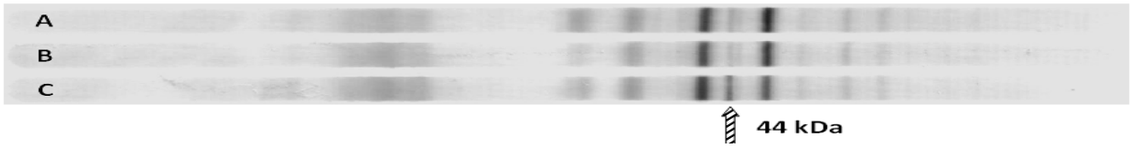


Fig. 10. Clinically successful treatment of facial palsy with serological progression (IgG)

3.2.6 Example 6

EM started on 07.06.2011., one day after the tick bite.

A, 23.06.2011. She visited our Centre with an EM 5cm in diameter and refused antibiotics since homeopathic treatment was thought to be safer.

B, 21.07.2011. The EM increased to 60cm. Faint serological progression was seen only in IgM. According to our in-house standards this result was only borderline.

Fig. 11. EM treated with homeopathy (IgM)

3.2.7 Example 7

EM started in September, 2010 disappeared without any treatment by the end of February, 2011.

A, 28.03.2011. Immunoblot showed very strong antibody reaction.

B, 04.08.2011. COMPASS suggested the survival of Bb in spite of that the patient remained free of symptom. Almost every band became more intense and some new bands appeared.

C, Positive control sample is also included for comparison of the band intensity.

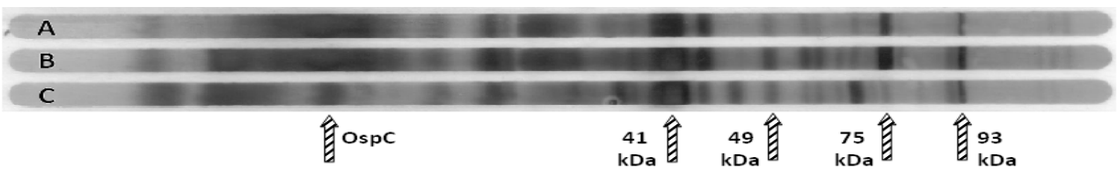


Fig. 12. Untreated erythema migrans (IgG)

3.3 COMPASS in possible relapse of Lyme borreliosis

3.3.1 Example 1

A, 06.07.2008. The patient visited our Centre with EM.

B, 14.06.2011. COMPASS showed serological regression in OspC while the other bands remained unchanged four months after the debut of arthralgia. Our decision was that the new complains are not related to the previous Bb infection.

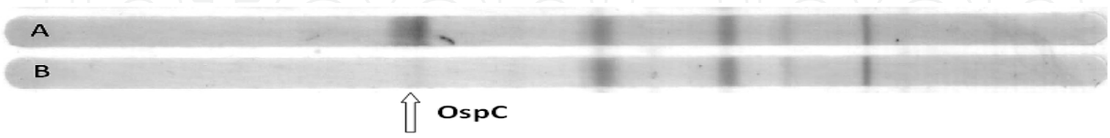


Fig. 13. No relapse (IgG)

3.3.2 Example 2

Between November 2009 and September 2010 this patient was treated with five courses of antibiotics in other institutes since he was found to be seropositive and had complains of paraesthesia, fatigue and abnormal cardiac palpitation. There was a probable EM in her history in around 1996.

A, 09.09.2010. There are several faint bands in this lane, suggesting the positive reaction is a result of immune memory but not an active Bb infection.

B, 12.05.2011. Eight months later the result is still „positive" but serological regression is visible in the 37kDa band. Actual Bb infection was ruled out.

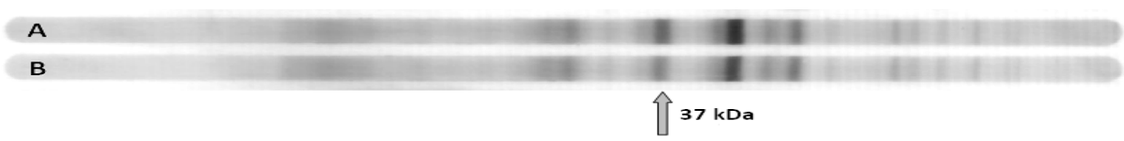


Fig. 14. No actual Lb in spite of the "positive" serological reaction (IgG)

3.4 COMPASS for screening in pregnancy after tick bite

3.4.1 Example 1

A, 08.06.2011. This sample was drawn at the 39th week of pregnancy, 17 days after a tick bite.

B, 27.06.2011. An erythema started and developed to a typical EM five days after the previous sampling. Minimal seroprogression is visible (striped arrows).

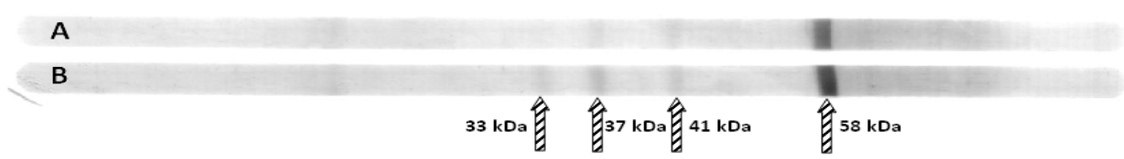


Fig. 15. Seroprogression with interval of 19 days (IgM)

3.4.2 Example 2

A, 23.06.2011. Sample of this patient with 14 weeks of pregnancy was drawn 17 days after a tick bite. There was no antibody response at this time.

B, 21.07.2011. Antibody response progressed definitively 28 days later. She was treated with ceftriaxone in spite of being free of symptoms.

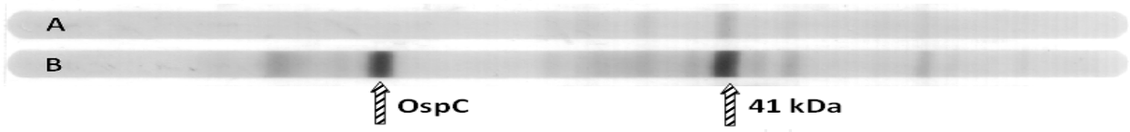


Fig. 16. Serological progression after a tick bite in pregnancy (IgG)

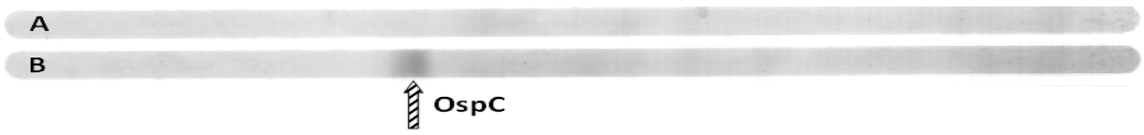


Fig. 17. Minimal amount of serological progression in IgM (same patient)

3.5 COMPASS for screening - after multiple tick bites

3.5.1 Example 1

A, 01.06.2011. This test was done after ten tick bites (30.05.2011.). We observed a borderline reaction.

B, 15.06.2011. This sample was drawn when an EM developed to 11cm in diameter.

Fig. 18. Seroprogression after 14 days (IgM)

3.6 COMPASS in atypical erythema migrans

3.6.1 Example 1

A, 15.06.2011. Rapidly progressing, significantly swollen, painful erythema shaped to 4cm after a probable tick bite (27.05.2011.). Typical EM 7cm in diameter developed eight days later.

B, 23.06.2011. The second sample is still negative but the serological progression is definitive.

Fig. 19. Seroprogression within 8 days (IgM)

3.7 COMPASS in facial palsy

3.7.1 Example 1

A, 17.01.2011. Facial palsy started in November 2010. The patient was treated with methylprednisolone and healed in four days. The result was in the negative range.

B, 23.03.2011. He came back two months later. Serological progression in the 21kDa band suggests active Bb infection, antibiotic treatment was started. This is a good example for that one band may decrease while another increase in intensity. That is why ELISA is not an appropriate method for comparative assay since the regression in a particular band may be compensated by the progression of another band.

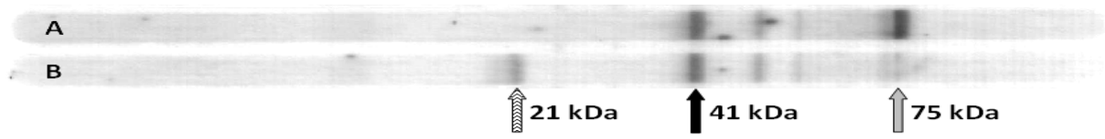


Fig. 20. Seroprogression in a symptom free patient after peripheral facial palsy (IgG)

3.8 COMPASS for evaluating intrathecal *Borrelia burgdorferi* s.l. antibody production

3.8.1 Example 1

There is a consensus regarding the demonstration of intrathecal antibody production as the currently most reliable diagnostic tool for Lyme disease with neurological involvement. Based on our study, intrathecal antibody production was considered to be positive if the immunoblot pattern observed clearly differed between the serum and the CSF sample in a pair. The difference could consist of bands present in CSF but not in serum or, conversely, bands that were present in serum were lacking in CSF, while at least one of the corresponding bands in the CSF was equally or more intense than in the serum. In addition to these differences, the intensity of the bands were also used as diagnostic criterion, if the difference was so disproportionate that a theoretical dilution or concentration of either the samples could not result in the same pattern as the other sample of the pair (Lakos et al, 2005).

In this example, there is a band (39kDa) in the CSF (C) missing from the serum (S) while an inverse situation is seen in the 24kDa band.

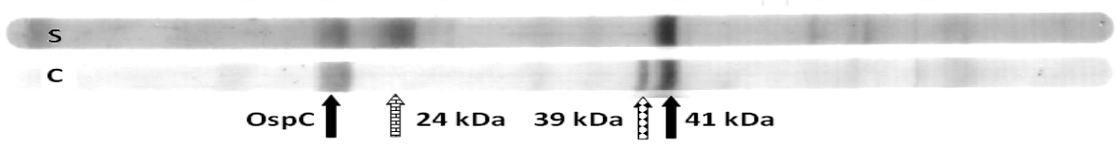


Fig. 21. Neuroborreliosis (lymphocytic meningoradiculits, Bannwarth's syndrome - IgM)

In IgG, there is a strong difference between serum and CSF in the 41 and the 93kDa bands, while the intensity of 47kDa almost the same in the serum and in the CSF. In this case both IgM and IgG represent the intrathecal Bb antibody synthesis, but either of them would fulfil the diagnostic criterion of proving the neuroborreliosis.

Fig. 22. Same patient (IgG)

3.8.2 Example 2

19.12.2010. Epilepsy developed in this forestry worker, and positive Bb antibody IgG reaction was found in the serum and CSF in another institute. In the CSF, only very faint bands are found, and they mirror the band pattern of the serum. There is no intrathecal antibody production, therefore neuroborreliosis is ruled out. For comparison, the positive control sample is included to illustrate that the bands of the forestry worker, although there are many, they are faint and dim. This is typical late sign for the previously healed long lasting or repeated Bb infection as it frequently seen in high risk patients.

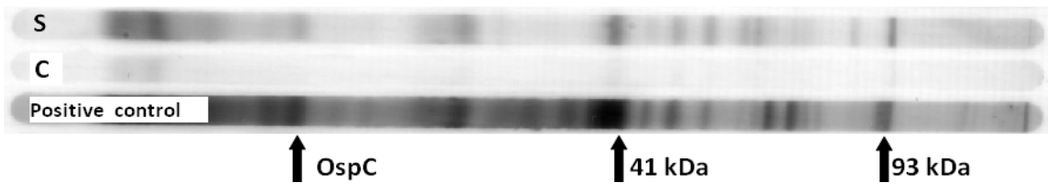


Fig. 23. Serum and CSF pair of a forestry worker (IgG). Neuroborreliosis is excluded.

3.8.3 Example 3

A, 28.11.2010. This forestry worker was tested because of isolated hypoglossal palsy started a year before the first sampling. Even there are definitive bands in the CSF (C) (an ELISA test was IgG positive in another institute), they wanly mirror the band pattern of the serum (S), therefore there is no sign of intrathecal Bb antibody synthesis, and we ruled out neuroborreliosis. He did not get antibiotic treatment.

B, 22.07.2011. The repeated COMPASS shows no change, supporting that our previous opinion was correct. The "positive" ELISA reaction originated from leakage of the blood-brain barrier.

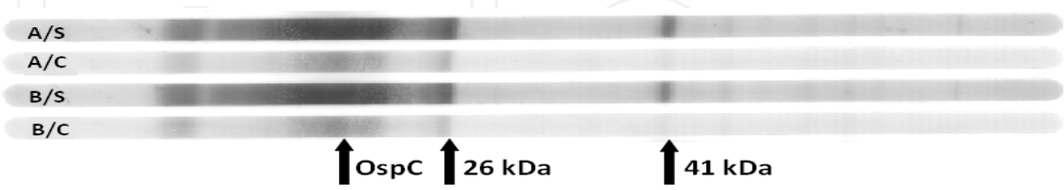


Fig. 24. Serum and cerebrospinal fluid pair of a forestry worker with positive reaction in both samples but without neuroborreliosis (IgG)

3.9 COMPASS in high risk patients

3.9.1 Example 1

A, 25.06.1992. He was first tested as a member of a survey among forestry workers. He was free of any symptoms at that time. The characteristic of WB (dim bands) and COMPASS applied with an interval of six months (not shown) suggested that Bb infection healed earlier.

B, 07.04.1999. Arthralgia started in 1999 but based on COMPASS, we ruled out the possibility of a present infection. In contrast, his samples were regularly tested as positive in other labs, and he was regularly treated with antibiotics. He received doxycycline and ceftriaxone courses ten times during the following 2 years.

C, 21.05.2002. In May 2002 his complains seriously worsened. Based on COMPASS, we definitely ruled out an active Borrelia infection but antibiotic treatments were still repeatedly prescribed by other physicians.

In March, 2003 osteosarcoma was disclosed and the patient died in September 2003.

Fig. 25. Serological follow up in a forestry worker with previously healed Bb infection died of osteosarcoma recognized too late (IgG)

3.9.2 Example 2

A, 07.07.2010. This symptom free forestry worker was screened and found to be positive in other institute. He was not treated with antibiotic. The dim bands suggested previous and long lasting but already healed infection. We repeated the test.

B, 16.05.2011. The unchanged band pattern of the second sample proved our original opinion: there is no actual Bb infection.

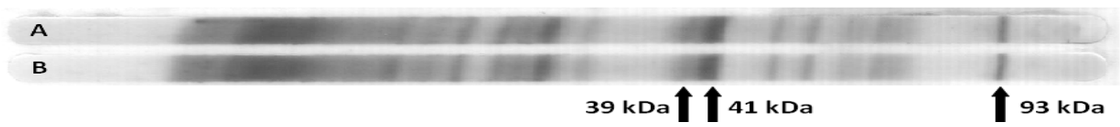


Fig. 26. No actual Bb infection in spite of the positive serological result (IgG)

3.10 COMPASS in reinfection

3.10.1 Example 1

A, 16.07.2008. This sample was drawn one month after 10 tick bites, but the result was negative at this time.

B, 29.06.2011. Almost three years later, the second sample was drawn 9 days after 40 spots of typical EM appeared as a result of new tick-bites. This example represent that multiple EM always present with extremely positive IgM reaction and this is shown at the very early stage of the clinical symptom.

Fig. 27. Intensive seroprogression in IgM following a multiple EM

3.10.2 Example 2

A, 20.10.2010. Parkinson's syndrome was started after an EM, two years before the first sampling. Based on the result, even positive, but faint and dim bands, chronic neuroborreliosis was ruled out.

B, 23.06.2011. The second sample was drawn on the day of recognition of a new EM with 6cm in diameter, 10 days after a tick bite. The serological progression was evident.

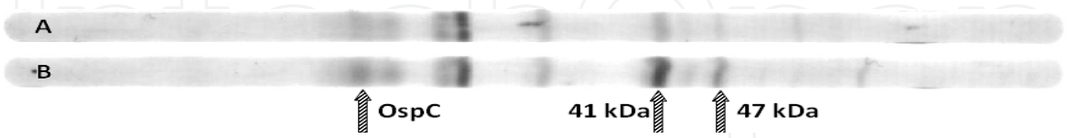


Fig. 28. Serological progression after reinfection (IgG)

3.10.3 Example 3

In this hunter, EM was started in September 2005. Healed, but 18 months later arthralgia developed.

A, 18.07.2007. The band pattern (dim and faint bands) was typical for already healed, previous Bb infection. Actual Lb was ruled out.

B, 15.06.2011. A giant EM, 100cm in diameter recognised one week ago. The band pattern is typical for reinfection: some of the previous bands disappeared but new bands presented. In the second sample, the bands are more intensive than they were in the previous sample.

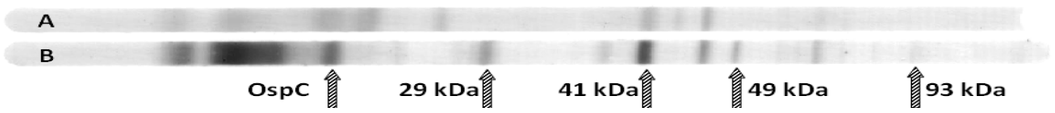


Fig. 29. Reinfection in a hunter (IgG)

3.10.4 Example 4

A, 31.08.2010. This patient was followed because of an earlier EM (May, 2010).

B, 04.04.2011. Three weeks before this sampling an itchy erythema developed with 15cm in diameter, tick bite was not recognised. The spot fainted a week ago. The serological progression in IgM proved that the patient has actual Bb infection and antibiotic was prescribed.

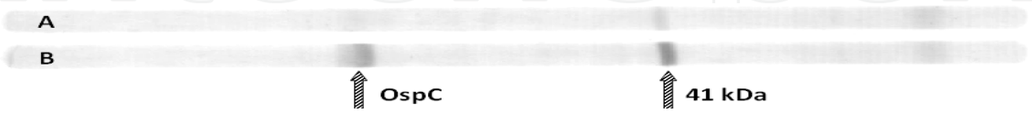


Fig. 30. Signs of reinfection (IgM)

3.11 COMPASS in probable false positive serological reaction

3.11.1 Example 1

This patient was examined because she had positive IgM Borrelia immunoblot results in other lab.

A, 23.05.2011. Our test also showed positive reaction in IgM but her symptom (eczema) did not suggest Lb. Therefore, we did not treat her with antibiotic in spite of the positive serological result.

B, 28.06.2011. Instead, we repeated the test. The COMPASS did not reveal progression in IgG neither changes in IgM. This suggests that the patient had false positive antibody reaction and actual Bb infection was ruled out.

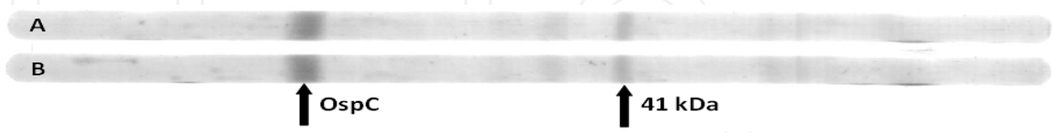


Fig. 31. Patient with false positive IgM reaction with no change in COMPASS

3.12 COMPASS for testing possible foetal Borrelia infection

3.12.1 Example 1

A, 23.06.2010. This patient is a pregnant woman with EM, in the end of first trimester. We treated her with ceftriaxone.

B, 24.01.2011. This sample was collected at the day of delivery.

C, 24.01.2011. Cord blood sample of the newborn. Minimal amount of serological regression is seen after 7 months. The band pattern of the newborn is exactly mirrors the band pattern of the mother. IgM was completely negative. Our opinion was that the foetus did not acquire Bb infection.

Fig. 32. COMPASS in maternal and newborn samples (IgG)

3.12.2 Example 2

Mother recognised her EM at her 32nd week of pregnancy. She was treated with ceftriaxone only for 11 days because of allergic reaction.

A, 29.06.2011. The mother still had a strong reaction in 41kDa IgM at the time of delivery.

B, 29.06.2011. But the newborn had no antibody in IgM (neither in IgG), representing that he did not get the infection. Note: IgM can not cross the placental barrier, therefore IgM antibodies in the newborn sample would mean foetal infection – but this was not the case.

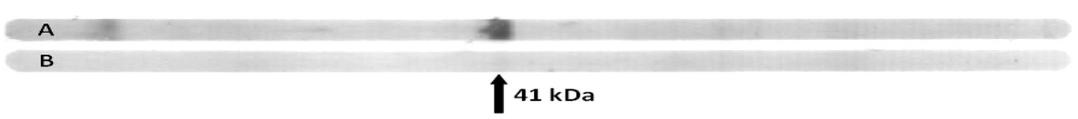


Fig. 33. After healing of the maternal EM, mother still has strong band in IgM, but the newborn has no antibody as a sign of that he has not been infected.

3.12.3 Example 3

This patient recognised an EM at the time of her twenty-fourth week of pregnancy.

A, 18.11.2009. This is a sample drawn three months after the treatment.

B, 15.12.2009. Cord blood sample. Even the newborn is also positive in IgG, all bands mirror the band pattern of the mother. The foetus has not been infected.

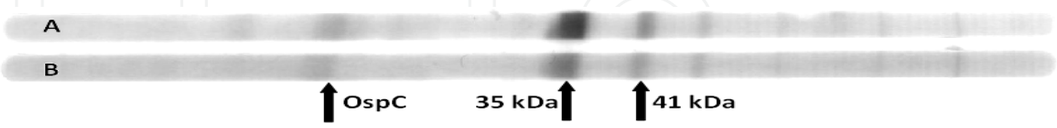


Fig. 34. Maternal and newborn samples (IgG)

4. Discussion

Here we presented a method which is useful for any laboratory working with WB, without any investment. We believe that this method significantly improve the serological diagnosis of Lb and can solve most of the problems may occur in this field. The method is basically simple, comparison of two samples tested side by side by WB. Testing two samples drawn with an interval we can prove the microbiological cure and this results in satisfaction of the patients and prevents abuse of antibiotics and repeated testing. Also we can find those patients who have persistent infection, after a retreatment their microbiological cure can be proven at the end.

Uncountable studies have published on serological diagnosis of Lb and many of them dealt with serological regression after treatment. We are not aware of a single paper on the description of the serological progression detected by Wb in treated or untreated Bb infected patients. We found only one similar study where Toxoplasma gondii WB of immunocompromised patients were studied in a similar setting as our COMPASS (Ashburn et al., 1998). Another work on untreated and spontaneously healed Lb patients was published but only the serological regression was detected by ELISA (Szer et al., 1991).

Since the discovery of Lb we are aware of that the infection may be generalized and became chronic, therefore the diagnosis of the disease is equal to the introduction of antibiotic treatment. This treatment aborts the antibody response. Therefore it is a rare occasion when we can observe the serological progression, the development of the antibody response in the course of time. This observation may improve in finding specific antigens. In contrast to the USA we have no consensus on which antibodies are the best for supporting the diagnosis of Lb in Europe (Robertson et al., 2000). It is obvious that the observation of the characteristics of serological progression could help to find the best antibodies for diagnostic purposes. If we can find antibodies progressing in the untreated patients in contrast to the controls, these should be considered specific. Our examples show that some specific antibodies decrease during the serological progression. We think this phenomenon is not generally known. Detecting the progressing antibodies in untreated patients may indicate of specificity irrespectively of that the result is otherwise "positive" or "negative". It should be stressed that the negative serological result does not rule out the possibility of Lb during the first two months of the infection, as positive result does not necessarily imply actual Bb infection since this can also reflect to a previous – many times asymptomatic - infection.

Consequently, testing only one sample is not enough for definitive diagnosis in many if not most case. In contrast, testing of a sample pair, the serological progression strongly supports the actual infection and the unchanged reaction represents the opposite. Ideally, a study on a regularly tested, untreated group of patients with clinically characteristic sign of Lb could answer that what the optimal interval is for drawing the sample pairs and which and how intensive antibodies appear during time. Since this is ethically nonsense, only the regular collection of these data on clinical and laboratory praxis can help.

Here we presented untreated patients where the amount of the antibodies and/or the number of the bands increased with time. It is clear that the serodiagnosis is more accurate when we compare the actual antibody response with a previous sample than with the cut-off. We presume that the serological progression proves the active, present infection (except some well defined situation, such as shortly after the antibiotic treatment). When the antibody profile is in the positive range but remains unchanged it means that the antibodies originate from a previous, already healed infection. COMPASS may increase the specificity of Bb antibody testing since it can discriminate past, healed and present, active infection. Confusion of these two possibilities of the positive reaction results in low PPV of serological tests. Application of COMPASS can avoid the problem of that different tests set up the cut-off levels in different ways.

The main topic of this study is to help physicians and microbiologists in those cases where the clinical picture is suggestive for Lb but ambiguous. It is generally accepted that clinical case definition of EM consists of at least 5cm in diameter, gradually enlarging rash. Morphea, tinea, Schamberg purpura, granuloma anulare, erythema nodosum, and allergic reaction to the tick itself may fulfil these criteria in particular cases. Not every clinician is sufficiently experienced to be able to distinguish these symptoms from EM and also, there are situations where competent clinicians also have to face diagnostic problems. Serology is the only tool for supporting the diagnosis in doubtful cases. Antibody response is regularly missing in the early cases. A few weeks later the progression in the antibody reaction can prove the Bb infection right before the serological result became “positive”.

We have to face confusion not only in cases with early symptoms suspect to Lb where neither clinical signs nor serology can help. An old forestry worker probably has strong antibody reaction to Bb and also complains of musculoskeletal symptom. Is there a causal relation? COMPASS can solve the problem: if the serological progression is missing in the untreated worker, Bb infection can be ruled out.

Every guideline emphasizes that Lb should be based on characteristic clinical symptoms, and serology is only a second line, supplementary test for supporting the clinical diagnosis (CDC, 1997). Moreover, guidelines also emphasize that serological confirmation does not need in cases of characteristic symptoms (e.g. EM). The bigger half of the Lb patients belongs to this group. Beyond EM, there are only very few patients whom pretest probability high for positive result of Bb antibody testing. For example, lymphocytic meningoradiculitis (LMR) with high protein and low glucose level in the CSF (Lakos, 1992), and facial palsy becoming bilateral in two weeks or with a tick bite behind the auricle are very suspicious for Bb infection (Lakos, unpublished), as well as fluctuating arthritis of the knee with impressive swelling but relatively moderate pain (Gerster et al., 1981; Shapiro & Gerber, 2000).

The number of these patients with high pretest probability is dwarfed by the total number of suspect cases. Oligoarthritis can be caused by many other illnesses, the etiology of facial palsy

is also divers. In most of the LMR cases the stiff neck is minimal (Ryberg, 1984; Sindic et al., 1987); therefore cerebrospinal fluid exam is rarely done. Moreover, most of the LMR patients are treated with antibiotics before the suspicion of Lb would arise, and this may abort the antibody response. Chronic Borrelia meningitis is rarely seen in the last decade. In spite of the guidelines' advice, most of the Bb antibody tests are done in the patients with low pretest probability for positive Bb antibody result (Coumou et al., 2011; Lakos et al., 2010).

The basic problem is that where the (i) pretest probability is high, evaluation of the clinical signs (e.g. EM) is more sensitive than serology, therefore antibody testing is not warranted, while in cases (ii) with low pretest probability (e.g. general symptoms, malaise, fatigue, polyneuropathy, arthralgia, myalgia) is again contraindicated just because of the low PPV (Lakos et al., 2010, Lakos & Igari, 2011). Following these rules, there would be a very few cases where Bb antibody test is reasonable. Why are so many tests done after all?

Half of the adult population complains of some kind of musculoskeletal symptoms in a nationwide US prevalence study (Lawrence et al., 2008). This means that Bb antibody testing may need with reason in everyone at least once in a period of life. Moreover, patients and their doctors would like to change the diagnosis of an incurable disease (e.g., multiple sclerosis, rheumatoid arthritis) to a curable one (i.e., Lb) or a disease of unknown origin to a disease with a clear-cut etiology. Therefore, there is a strong pressure on doctors to test for Bb antibodies in patients suffering from diseases of serious course and/or of unknown origin. This pressure leads to the substantial consumption of Bb antibody tests. Only at the Mayo Clinic more than 75.000 tests were done in a year (Binnicker et al., 2008). The more tests are consumed the more false positive results are expected.

If the clinical sign is not typical for Lb, a single positive serological result must not be accepted as a proof of Bb infection and must not be followed by antibiotic treatment. Instead, a second sample should be drawn after an interval, and then not the positivity but the tendency of the antibody production, i.e. presence or absence of the progression will provide definitive result and strong support for the clinician whether the treatment is indicated or not. Applying COMPASS in those cases where the clinical signs are not typical for Lb may decrease the false diagnoses and antibiotic abuse.

Only Wb is appropriate for the comparative test, since progression in one or two antibodies can be faded by the total amount of antibody response. (ELISA measures the whole amount of antibodies; therefore only massive difference between the sample pair may be evaluable with this method). Parallel examination of the sample pair is also important since the intralaboratory fluctuation of the results can be more intense than the real change in the patient.

5. Summary

COMPASS

a. May help the clinician in cases suspicious for EM but the clinical diagnosis is not clean cut. A blood sample should be drawn at the first visit, and if the rash does not develop into a typical form, wait for collecting the second sample instead of antibiotic treatment. Than the sample pair is examined in parallel and the presence / absence of the progression provide a definitive judgment whether the patient acquired Bb infection or not.
b. It is useful for proving the microbiological cure.

c. It is useful for determining the diagnosis when the first sample reveals positive antibody response but the clinical symptoms are not specific for Lb.
d. It is appropriate for the demonstration of the intrathecal Borrelia antibody production. In this setting the assay is completed with serum and CSF pair drawn at the same day (Lakos et al., 2005).
e. It is useful in distinguishing present and past Bb infection in high risk group patients (forestry workers, orienteers, hunters, etc.) where the positive serological reaction is quite frequent but Lb with clinical symptoms is relatively rare (Lakos 2011).
f. It is appropriate for proof /exclusion of the persistent, relapsed and repeated infections.
g. It may be appropriate for comparison of different antibiotic treatment / regime in such a disease (i.e. EM) where the spontaneous clinical improvement is frequent.
h. It is probably appropriate for disclosing materno-fetal transmission of Bb in cases of Lb during pregnancy.

COMPASS would probably help in other infection where serology is routinely applied but present and past infection can not be distinguished easily (e.g. Toxoplasma gondii, Chlamydophila pneumoniae etc.).

6. References

Ackermann, R.; Hörstrup, P. & Schmidt, R. (1984). Tick-borne meningoneuritis (Garin-Bajadoux, Bannwarth). *The Yale Journal of Biology and Medicine,* Vol.57, No.4, (July-August 1984), pp. 485-490

Andrew, M. A.; Easterling, T. R.; Carr, D. B; Shen, D.; Buchanan, M. L.; Rutherford, T; Bennett, R.; Vicini, P. & Herbert, M. F. (2007). Amoxicillin pharmacokinetics in pregnant women: modeling and simulations of dosage strategies. *Clinical Pharmacology and Therapeutics*, Vol.81, No.4, (April 2007), pp. 547–556

Ang, C.W.; Notermans, D.W.; Hommes, M.; Simoons-Smit, A. & Herremans, T. (2011). Large differences between test strategies for the detection of anti-Borrelia antibodies are revealed by comparing eight ELISAs and five immunoblots. *European Journal of Clinical Microbiology & Infectious Diseases,* Vol.30, No.8, (August 2011), pp. 1027-1032

Arnez, M.; Pleterski-Rigler, D.; Luznik-Bufon, T.; Ruzić-Sabljić, E. & Strle, F. (2002). Solitary erythema migrans in children: comparison of treatment with azithromycin and phenoxymethylpenicillin. *Wiener Klinische Wochenschrift,* Vol.114, No.13-14, (July 2002), pp. 498-504

Ashburn, D.; Davidson, M. M.; Joss, A. W.; Pennington, T. H. & Ho-Yen, D. O. (1998). Improved diagnosis of reactivated toxoplasmosis. *Molecular Pathology,* Vol.51, No.2, (April 1998), pp.105-109

Bacon, R.M.; Biggerstaff, B.J.; Schriefer, M.E.; Gilmore, R.D. Jr; Philipp, M.T..; Steere, A.C.; Wormser, G.P.; Marques, A.R. & Johnson, B.J. (2003). Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of Borrelia burgdorferi compared with 2-tiered testing using whole-cell lysates. *The Journal of Infectious Diseases,* Vol.187, No.8, (April 2003), pp. 1187–99

Banyas, G. T. (1992). Difficulties with Lyme serology. *Journal of the American Optometric Associaion,* Vol.63, No.2, (February 1992), pp. 135-139

Baradaran-Dilmaghani, R. & Stanek, G. (1996). In vitro susceptibility of thirty Borrelia strains from various sources against eight antimicrobial chemotherapies. *Infection,* Vol.24, No.1, (January-February 1996), pp. 60–63

Binnicker, M.J.; Jespersen, D.J.; Harring, J.A.; Rollins, L.O.; Bryant, S.C. & Beito, E. M. (2008). Evaluation of two commercial systems for automated processing, reading, and interpretation of Lyme borreliosis Western blots. *Journal of Clinical Microbiology,* Vol.46, No.7, (July 2008), pp. 2216-2221

Burgdorfer, W.; Barbour, A. G.; Hayes, S. F.; Benach, J. L.; Grunwaldt, E. & Davis, J. P. (1982). Lyme disease-a tick-borne spirochetosis? *Science,* Vol.216, No.4552, (June 1982), pp. 1317-1319

Coumou, J.; van der Poll, T.; Speelman, P. & Hovius, J.W. (2011). Tired of Lyme borreliosis. Lyme borreliosis in the Netherlands. *The Netherlands Journal of Medicine,* Vol.69, No.3, (March 2011), pp. 101-111

Centers for Disease Control and Prevention. (1995). Recommendations for Test Performance and Interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR Morbidity and Mortality Weekly Report,* Vol.44, No.31, (August 1995), pp. 590-591

Centers for Disease Control and Prevention. (1997). Case definitions for infectious conditions under public health surveillance. *MMWR Recommandations and Reports,* Vol.46, No.10, (May 1997), pp. 1-55

Centers for Disease Control and Prevention. (2008). Lyme Disease (Borrelia burgdorferi) 2008 Case definition. In: www.cdc.gov, 01.09.2011, Available from: http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/lyme_disease_2008.htm

Craft, J. E.; Grodzicki, R. L.; Shrestha, M.; Fischer, D. K.; García-Blanco, M. & Steere, A. C. (1984). The antibody response in Lyme disease. *The Yale Journal of Biology and Medicine,* Vol.57, No.4, (July-August 1984), pp. 561-565

Cutler, S. J. & Wright, D. J. (1994). Predictive value of serology in diagnosing Lyme borreliosis. *Journal of Clinical Pathology,* Vol.47, No.4, (April 1994), pp. 344-349

Donta, S.T. (1997). Tetracycline therapy for chronic Lyme disease. *Clinical Infectious Diseases,* Vol.25, Suppl.1, (July 1997), pp. 52-56

Dressler, F.; Whalen, J. A.; Reinhardt, B. N. & Steere, A. C. (1993). Western blotting in the serodiagnosis of Lyme disease. *The Journal of Infectious Diseases,* Vol.167, No.2, (February 1993), pp. 392–400

Gerster, J. C.; Guggi, S.; Perroud, H. & Bovet, R. (1981). Lyme arthritis appearing outside the United States: a case report of Switzerland. *British Medical Journal,* Vol.283, No.6297, (October 1981), pp. 951-952

Goettner, G.; Schulte-Spechtel, U.; Hillermann, R.; Liegl, G.; Wilske, B. & Fingerle, V. (2005). Improvement of Lyme borreliosis serodiagnosis by a newly developed recombinant immunoglobulin G (IgG) and IgM line immunoblot assay and addition of VlsE and DbpA homologues. *Journal of Clinical Microbiology.* Vol.43, No.8, (August 2005), pp. 3602-3609

Halperin, J. J., Volkman, D. J. & Wu, P. (1991). Central nervous system abnormalities in Lyme neuroborreliosis. *Neurology,* Vol.41, No.10, (October 1991), pp. 1571-1582

Hauser, U.; Lehnert, G.; Lobentanzer, R. & Wilske, B. (1997). Interpretation criteria for standardized Western blots for three European species of Borrelia burgdorferi sensu lato. *Journal of Clinical Microbiology,* Vol.35, No.6, (June 1997), pp. 1433-1444

Hunfeld, K. P.; Ruzic-Sabljic, E.; Norris, D. E.; Kraiczy, P. & Strle, F. (2005). In vitro susceptibility testing of Borrelia burgdorferi sensu lato isolates cultured from patients with erythema migrans before and after antimicrobial chemotherapy. *Antimicrobial Agents and Chemotherapy,* Vol.49, No.5, (April 2005), pp. 1294-1301

Kaiser, R. (2000). False-negative serology in patients with neuroborreliosis and the value of employing of different borrelial strains in serological assays. *Journal of Medical Microbiology,* Vol.49, No.10, (October 2000), pp. 911-915

Kannian, P.; McHugh, G.; Johnson, B. J.; Bacon, R. M.; Glickstein, L. J. & Steere, A. C. (2007). Antibiotic responses to Borrelia burgdorferi in patients with antibiotic-refractory, antibiotic-responsive, or non-antibiotic-treated Lyme arthritis. *Arthritis and Rheumatism,* Vol.56, No.12, (December 2007), pp. 4216-4225

Karlsson, M.; Hammers, S; Nilsson-Ehle, I.; Malmborg, A. S. & Wretlind, B. (1996) Concentrations of doxycycline and penicillin G in sera and cerebrospinal fluid of patients treated for neuroborreliosis. *Antimicrobial Agents and Chemotherapy,* Vol.40, No.5, (May 1996), pp. 1104-1107

Kleibeuker, W.; Zhou, X.; Centlivre, M.; Legrand, N.; Page, M.; Almond, N.; Berkhout, B. & Das, A. T. (2009) A sensitive cell-based assay to measure the doxycycline concentration in biological samples. *Human Gene Therapy,* Vol.20, No.5 (May 2009), pp. 524-530

Klempner, M. S.; Hu, L. T.; Evans, J.; Schmid, C. H.; Johnson, G. M.; Trevino, R. P.; Norton, D.; Lew, L.; Wall, D.; McCall, J.; Kosinksi, M. & Weinstein, A. (2001). Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. *The New England Journal of Medicine,* Vol.45, No.2, (July 2001), pp. 85–92

Lakos, A. (1990). Comparison of four serological tests for Borrelia burgdorferi in Bell's palsy. *Seroiagnosis and Immunotherapy,* Vol.4, No.4, (August 1990), pp. 271-275

Lakos, A. (1992). Cerebrospinal findings in Lyme meningitis. *The Journal of Infection,* Vol.25, No.2, (September 1992), pp. 1-12

Lakos, A. & Granström, M. (1997). Diagnostic power of immunoblot in Lyme borreliosis. *Proceedings of The 15th Annual meeting of the European Society for Paediatric Infectious Disease,* Paris, France, May, 1997

Lakos, A.; Ferenczi, E.; Komoly, S. & Granström, M. (2005). Different B-cell populations are responsible for the peripheral and intrathecal antibody production in neuroborreliosis. *International Immunology*, Vol.17, No.12, (December 2005), pp. 1631-1637

Lakos, A.; Nagy, G. & Deák, L. C. (2008). Evaluation of the serological progression (COMPASS) in Lyme borreliosis (Hungarian). *Medicus Anonymus,* Vol.16, No.5-6. (May 2008), pp.20-21

Lakos, A. & Solymosi, N. (2010). Maternal Lyme borreliosis and pregnancy outcome. *The International Journal of Infectious Diseases,* Vol.14, No.6, (June 2010), pp. e494-498

Lakos, A.; Reiczigel, J. & Solymosi, N. (2010). The positive predictive value of Borrelia burgdorferi serology in the light of symptoms of patients sent to an outpatient service for tick-borne diseases. *Inflammation Research,* Vol.59, No.11, (November 2010), pp. 959-964

Lakos, A. & Igari, E. (2011). Clinical and serological survey for Lyme borreliosis among forestry workers (Hungarian). *Háziorvos Továbbképző Szemle,* Vol.16, (2011), pp. 10-12

Lawrence, R.C.; Felson, D.T.; Helmick, C.G.; Arnold, L.M.; Choi, H.; Deyo, R.A.; Gabriel, S.; Hirsch, R.; Hochberg, M.C.; Hunder, G.G.; Jordan, J.M.; Katz, J.N.; Kremers, H.M. & Wolfe, F. (National Arthritis Data Workgroup). (2008). Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. *Arthritis and Rheumatism,* Vol.58, No.1, (January 2008), pp. 26–35

Marangoni, A.; Sparacino, M.; Cavrini, F.; Storni, E.; Mondardini, V.; Sambri, V. & Cevenini, R. (2005). Comparative evaluation of three different ELISA methods for the diagnosis of early culture-confirmed Lyme disease in Italy. *Journal of Clinical Microbiology,* Vol.54, No.4, (April 2005), pp. 361-367

Maraspin, V.; Lotric-Furlan, S. & Strle, F. (2002). Development of erythema migrans in spite of treatment with antibiotics after a tick bite. *Wiener Klinische Wochenschrift,* Vol.114, No.13-14, (July 2002), pp. 616-619

Marques, A. (2008). Chronic Lyme disease: a review. *Infectious Disease Clinics of North America,* Vol.22, No.2, (June 2008), pp. 341-360

Nohlmans, M. K.; Blaauw, A. A.; van den Bogaard, A. E. & van Boven, C. P. (1994). Evaluation of nine serological tests for diagnosis of Lyme borreliosis. *Europian Jounal of Clinical Microbiology and Infectious Diseases,* Vol.13, No.5, (May 1994), pp. 394-400

Numthavaj, P.; Thakkinstian, A.; Dejthevaporn, C. & Attia, J. (2011). Corticosteroid and antiviral therapy for Bell's palsy: a network meta-analysis. *BMC Neurology,* Vol.11, (January 2011), pp. 1

Pachner, A. R. & Steere, A. C. (1984). Neurological findings of Lyme disease. *The Yale Journal of Biology and Medicine,* Vol.57, No.4, (July-August 1984), pp. 481-483

Porwancher, R.B.; Hagerty, C.G.; Fan, J.; Landsberg, L.; Johnson, B.J.; Kopnitsky, M.; Steere, A.C.; Kulas, K. & Wong, S.J. (2011). Multiplex immunoassay for Lyme disease using VlsE1-IgG and pepC10-IgM antibodies: improving test performance through bioinformatics. *Clinical and Vaccine Immunology,* Vol.18, No.5, (May 2011), pp. 851-859

Raoult, D., Hechemy, K. E. & Baranton, G. (1989). Cross-reaction with Borrelia burgdorferi antigen of sera from patients with human immunodeficiency virus infection, syphilis, and leptospirosis. *Journal of Clinical Microbiology,* Vol.27, No.10, (October 1989), pp. 2152-2155

Rath, P. M.; Marsch, W. C., Brade, V. & Fehrenbach, F. (1994). Serological distinction between syphilis and Lyme borreliosis. *Zentralblatt für Mikrobiologie,* Vol.280, No.3, (January 1994), pp. 319-324

Robertson, J.; Guy, E.; Andrews, N.; Wilske, B.; Anda, P.; Granström, M., Hauser, U.; Moosmann, Y; Sambri, V.; Schellekens, J. & Stanek, G. (2000). A European multicenter study of immunoblotting in serodiagnosis of Lyme borreliosis. *Journal of Clinical Microbiology*, Vol.38, No.6, (June 2000), pp. 2097–2102

Ryberg, B. (1984). Bannwarth's syndrome (lymphocytic meningoradicultis) in Sweden. *The Yale Journal of Biology and Medicine.* Vol.57, No.4, (July-August 1984), pp. 499-503

Shapiro, E. D. & Gerber, M. A. (2000). Lyme disease. *Clinical Infectious Disease,* Vol.31, No.2, (August 2000), pp. 533-542

Sindic, C. J.; Depre, A.; Bigaigon, G.; Goubau, P., F.; Hella, P. & Laterre, C. (1987). Lymphocytic meningoradiculitis and encephalomyelitis due to Borrelia burgdorferi: a clinical and serological study of 18 cases. *Journal of Neurology, Neurosurgery, and Psychiatric.* Vol.50, No.12, (December 1987), pp. 1565-1571

Stanek G, O'Connell S, Cimmino M, Aberer E, Kristoferitsch W, Granström M, Guy E, Gray J. (1996). European Union Concerted Action on Risk Assessment in Lyme Borreliosis: clinical case definitions for Lyme borreliosis. *Wiener Klinische Wochenschrift,* Vol.108, No.23, (December, 1996), pp.741-747.),

Stanek, G. & Strle, F. (2003). Lyme borreliosis. *Lancet,* Vol.362, No.9396, (November 2003), pp. 1639-1647

Steere, A. C.; Coburn, J. & Glickstein, L. (2004). The emergence of Lyme disease. *The Journal of Clinical Investigation,* Vol.113, No.8, (April 2004), pp. 1093-1101

Steere, A. C. & Angelis, S. M. (2006). Therapy of Lyme arthritis: strategies for the treatment of antibiotic-refractory arthritis. *Arthritis and Rheumatism,* Vol.54, No.10, (October 2006), pp. 3079-3086

Steere, A.C.; McHugh, G.; Damle, N. & Sikand, V.K. (2008). Prospective study of serologic tests for Lyme disease. *Clinical Infectious Diseases,* Vol.47, No.2, (July 2008), pp.188–195

Sullivan, F. M., Swan, I. R.; Donnan, P. T., Morrison, J. M.; Smith, B. H.; McKinstry, B.; Davenport, R.J.; Vale, L. D.; Clarkson, J. E., Hammersley, V.; Hayavi, S.; McAteer, A.; Stewart, K. & Daly, F. (2007). Early treatment with prednisolone or acyclovir in Bell's palsy. *The New England Journal of Medicine,* Vol.357, No.16, (October 2007), pp. 1598-1607

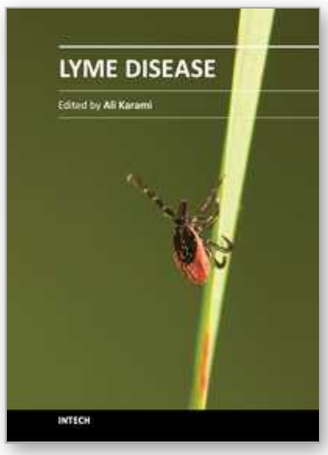
Szer, I. S.; Taylor, E. & Steere, A. C. (1991). The long-term course of Lyme arthritis in children. *The New England Journal of Medicine,* Vol.325, No.3, (July 1991), pp. 159-163

Tuuminen, T.; Hedman, K.; Söderlund-Venermo, M. & Seppälä, I. (2011). Acute parvovirus B19 infection causes nonspecificity frequently in Borrelia and less often in Salmonella and Campylobacter serology, posing a problem in diagnosis of infectious arthropathy. *Clinical and Vaccine Immunology,* Vol.18, No.1, (January 2011), pp. 167-172

Wilske, B., Schierz, G., Preac-Mursic, V., von Busch, K.; Kühlbeck, R.; Pfister, H. W. & Einhäuple, K. (1986). Intrathecal production of specific antibodies against Borrelia burgdorferi in patients with lymphocytic meningoradiculitis (Bannwarth's syndrome). The Journal of Infectious Diseases, Vol.153, No.2, (February 1986), pp. 304-314

Wormser, G. P.; Ramanathan, R.; Nowakowski, J.; McKenna, D.; Holmgren, D.; Visintainer, P.; Dornbush, R.; Singh, B. & Nadelman, R. B. (2003). Duration of antibiotic therapy for early Lyme disease. A randomized, double-blind, placebo-controlled trial. *Annals of Internal Medicine,* Vol.138, No.9, (May 2003), pp. 697-704

Wormser, G. P.; Dattwyler, R. J.; Shapiro, E. D.; Halperin, J. J.; Steere, A. C.; Klempner, M. S.; Krause, P. J.; Bakken, J. S.; Strle, F.; Stanek, G.; Bockenstedt, L.; Fich, D.; Dumler, J. S. & Nadelman, R. B. (2006). The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clinical Infectious Diseases.* Vol.43, No.9, (November 2006), pp. 1089-1134



Lyme Disease
Edited by Dr. Ali Karami

ISBN 978-953-51-0057-7
Hard cover, 160 pages
Publisher InTech
Published online 17, February, 2012
Published in print edition February, 2012

Lyme disease, or Lyme borreliosis, is an emerging infectious disease caused by bacteria belonging to the genus borrelia. Borrelia burgdorferi, in the strict sense. This book deals mostly with the molecular biology of the Lyme disease agent orrelia burgdorferi. It has been written by experts in the relevant field and is tailored to the need of researchers, advanced students of biology, molecular biology, molecular genetics of microorganism. It will also be of use to infectious disease experts and people in other disciplines needing to know more about Lyme borreliosis. The book contains chapters on the molecular biology of the Lyme disease agent, zoonotic peculiarities of Bb, advancement in Bb antibody testing, the serology diagnostic schemes in Bb, discovering Lyme disease in ticks and dogs, adaptation to glucosamine starvation in Bb, and porins in the genus borrelia.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

András Lakos and Erzsébet Igari (2012). Advancement in Borrelia burgdorferi Antibody Testing: Comparative Immunoblot Assay (COMPASS), Lyme Disease, Dr. Ali Karami (Ed.), ISBN: 978-953-51-0057-7, InTech, Available from: http://www.intechopen.com/books/lyme-disease/advancement-in-borrelia-burgdorferi-antibody-testing-comparative-immunoblot-assay-compass-

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen