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V. Preac Mursic, W. Marget, U. Busch, D. Pleterski Rigler, S. Hagl

Kill Kinetics of *Borrelia burgdorferi* and Bacterial Findings in Relation t the Treatment of Lyme Borreliosis*

Summary: For a better understanding of the persistence of *Borrelia burgdorferi sensu lato* (s.l.) after antibiotic therapy the kinetics of killing *B. burgdorferi s.l.* under amoxicillin, doxycycline, cefotaxime, ceftriaxone, azithromycin and penicillin G were determined. The killing effect was investigated in MKP medium and human serum during a 72 h exposure to antibiotics. Twenty clinical isolates were used, including ten strains of *Borrelia afzelii* and ten strains of *Borrelia garinii*. The results show that the kinetics of killing borreliae differ from antibiotic to antibiotic. The killing rate of a given antibiotic is less dependent on the concentration of the antibiotic than on the reaction time. Furthermore, the data show that the strains of *B. afzelii* and *B. garinii* have a different reaction to antibiotics used in the treatment of Lyme borreliosis and that different reactions to given antibiotics also exist within one species. The *B. garinii* strains appear to be more sensitive to antibiotics used in therapy. Furthermore, the persistence of *B. burgdorferi s.l.* and clinical recurrences in patients despite seemingly adequate antibiotic treatment is described. The patients had clinical disease with or without diagnostic antibody titers to *B. burgdorferi*.

Introduction

The diagnosis of Lyme borreliosis is based on clinical assessment and the detection of specific antibodies. However, interpretation of serological tests and results may not be straightforward, because such antibodies may persist for months and can indicate prior exposure to *Borrelia* as well as active disease. Negative serologic results do not necessarily exclude *Borrelia* infection [1–4].

Cultivation of *Borrelia* from CSF, organ and joint biopsies is difficult and relatively seldom done; the PCR method (polymerase chain reaction) has not yet been sufficiently evaluated for routine diagnosis of human specimens. Especially the diagnosis of late manifestations and persistence of the disease can be problematic.

Also the treatment of Lyme borreliosis is still associated with several unanswered questions. One of the main problems is the establishment of the optimal antibiotic therapy. The results of the comparative *in vitro* and *in vivo* studies of the susceptibility of *Borrelia burgdorferi sensu lato* (*s.l.*) demonstrate the sensitivity of borreliae to a number of antibiotics [5–9]. However, some patients developed symptoms of the disease later despite antibiotic treatment [10, 1–4, 11, 12].

In the present report the results of the kill kinetics of *B. burgdorferi s.l.* as well as the persistence of borreliae after antibiotic treatment are discussed.

Kill Kinetics of *Borrelia burgdorferi s.l.* under Various Antibiotics

Materials and Methods

Antibiotic susceptibility of *B. burgdorferi s.l.* and minimal inhibitory concentration (MIC_{90}) of 30 antibiotics have been reported previously [7, 8].

The kill kinetics of borreliae under various antibiotics was dete mined in MKP medium [13] and in undiluted volunteer seru with negative Lyme borreliosis serological tests. The *Borrelia g rinii* (ten strains) and *Borrelia afzelii* (ten strains) used in th study were isolated from human spinal fluid and skin biopsy. Th tubes of the MKP medium or serum containing antibiotic in co centrations of 1, 2 and 4 mg/l were inoculated with 10^7 cells/n Control tubes with 10^7 cells/ml but no antibiotics were include in every run. Incubation took place at 33°C. The cultures we examined for motile and non-motile borreliae by dark field m croscopy after 6, 12, 24, 48 and 72 h of incubation and by subcu tures. Subcultures in MKP medium without antibiotics we made and observed for growth for a further 4 weeks. The agen tested included amoxicillin, azithromycin, cefotaxime, ceftria one, doxycycline and penicillin G.

Results

Figures 1–5 illustrate the data on killing rates in *B. afze* species, Figures 6–10 on ten isolates of *B. garinii* species MKP medium. The results show that the kill kinetics of th borreliae differs from antibiotic to antibiotic. The killi rate of a given antibiotic for borreliae is less dependent the concentration of the antibiotic than on the reaction tim Furthermore, the data show that the killing effect of isolat of *B. garinii* differs from that in *B. afzelii* species. Very inte esting and unexpected is the different effect of antibiotics

Vera Preac Mursic, Ph. D., *U. Busch*, Dipl.-Biol., Max v. Pettenkofer stitut, Ludwig-Maximilians-Universität München, Pettenkoferstr. D–80336 München; *W. Marget*, M. D., Kinderklinik, Ludwig-Maxi lians-Universität München, Lindwurmstr. 4, D–80337 München, Ger ny; *Dusica Pleterski Rigler*, M. D., Dept. of Infectious Diseases, Univ sity Ljubljana, Japljeva 2, 61000 Ljubljana, Slovenia; *S. Hagl*, M. D., C rurgische Klinik, RK-Universität Heidelberg, Im Neuenheimer Feld 1 D–69120 Heidelberg, Germany.

***This article is the expanded version of a lecture delivered in Portoro 14 May 1995. See also page 59.**

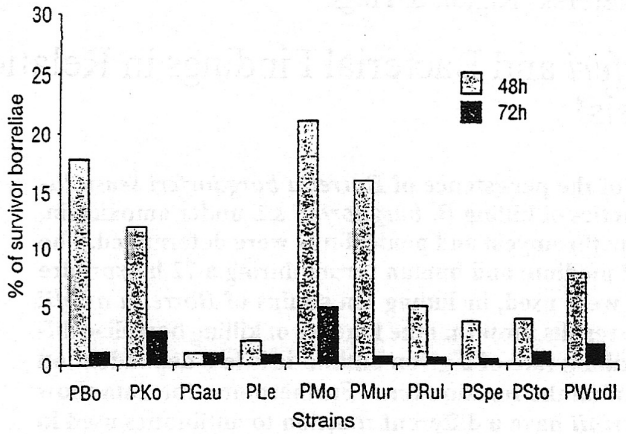

Figure 1: Kill kinetics at 48 and 72 h: MKP amoxicillin 4 mg/l; *Borrelia afzelii* strains.

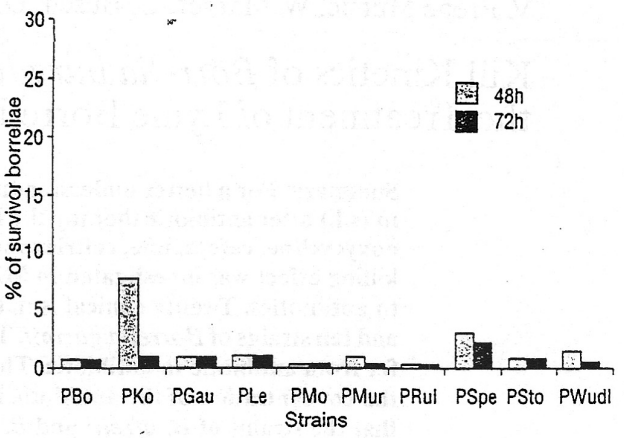

Figure 2: Kill kinetics at 48 and 72 h: MKP doxycycline 4 mg/l; *Borrelia afzelii* strains.

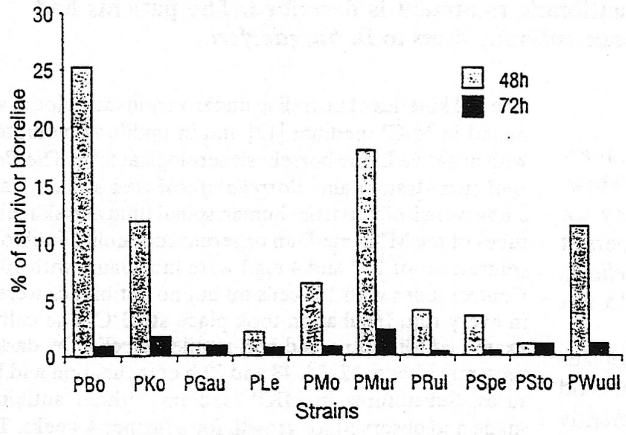

Figure 3: Kill kinetics at 48 and 72 h: MKP cefotaxime 1 mg/l; *Borrelia afzelii* strains.

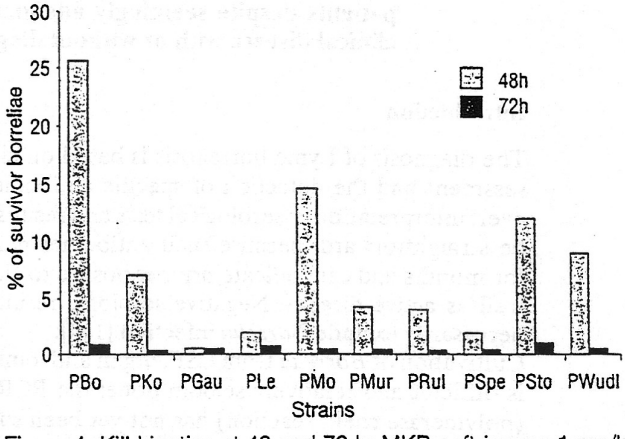

Figure 4: Kill kinetics at 48 and 72 h: MKP ceftriaxone 1 mg/l; *Borrelia afzelii* strains.

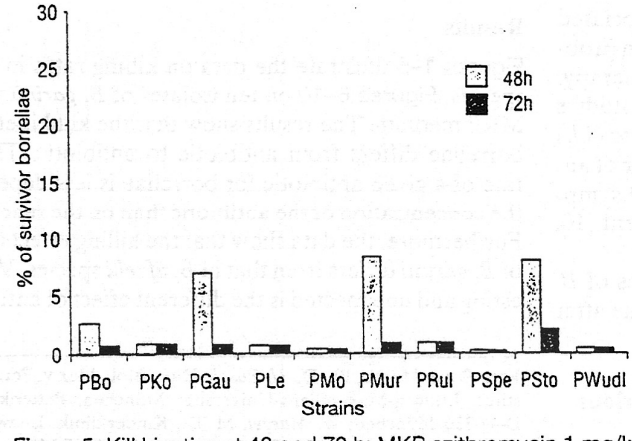

Figure 5: Kill kinetics at 48 and 72 h: MKP azithromycin 1 mg/l; *Borrelia afzelii* strains.

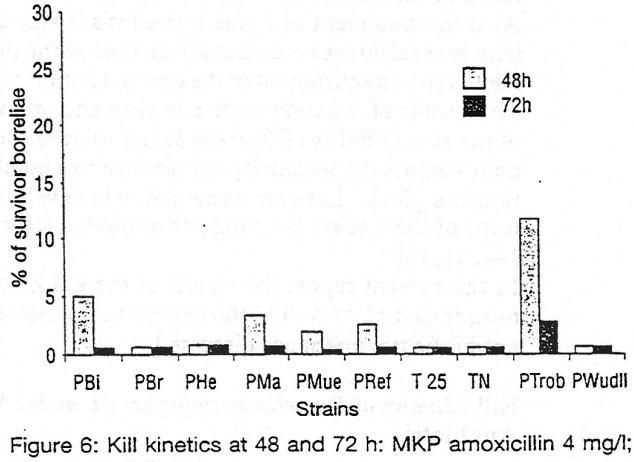

Figure 6: Kill kinetics at 48 and 72 h: MKP amoxicillin 4 mg/l; *Borrelia garinii* strains.

the isolates within one species. Also the different reaction of one strain to tested antibiotics is surprising.

To find out the antiborrelial activity of serum, and simultaneously the effect of serum and antibiotics against *Borrelia* isolates, we examined the effect of antibiotics in human serum. As shown (Figures 11, 12), the efficacy of antibiotics in volunteer serum is not significantly better than in MKP medium. Therefore serum did not significantly en-

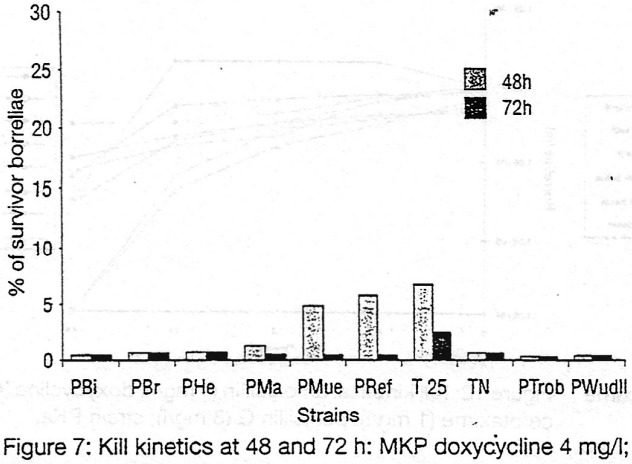

Figure 7: Kill kinetics at 48 and 72 h: MKP doxycycline 4 mg/l; *Borrelia garinii* strains.

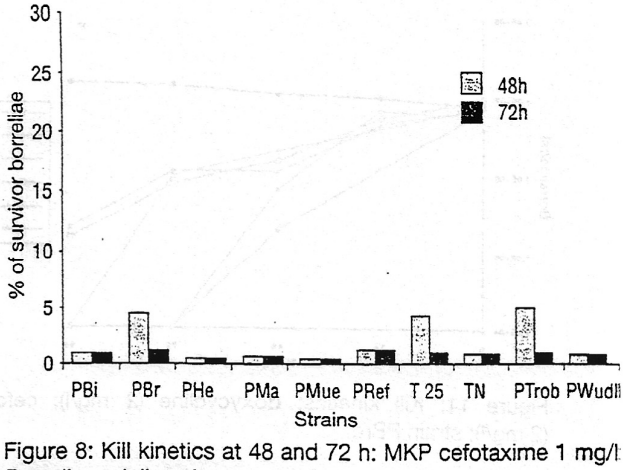

Figure 8: Kill kinetics at 48 and 72 h: MKP cefotaxime 1 mg/l *Borrelia garinii* strains.

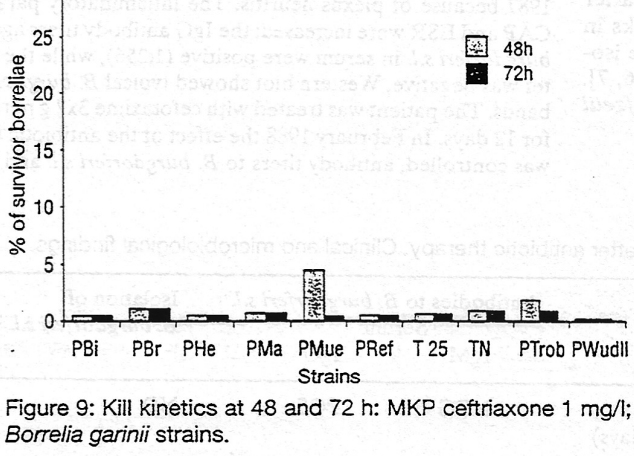

Figure 9: Kill kinetics at 48 and 72 h: MKP ceftriaxone 1 mg/l; *Borrelia garinii* strains.

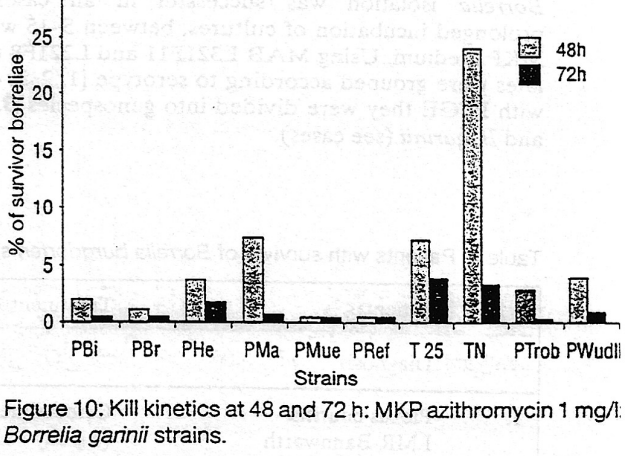

Figure 10: Kill kinetics at 48 and 72 h: MKP azithromycin 1 mg/l *Borrelia garinii* strains.

hance the bactericidal activity of the tested antibiotics and the activity was not reduced in the presence of serum.

In summary, the results of killing kinetics suggest that:

1. The strains of *B. afzelii* and *B. garinii* spp. react differently against antibiotics used in the treatment of Lyme disease.
2. The different reactions of strains to antibiotics also exist within one species.
3. There exist different effects of one antibiotic against strains tested as well as different reactions of the strain to antibiotics tested.
4. The killing rate of a given antibiotic is dependent on reaction time of antibiotics.
5. *B. garinii* strains seem to be more sensitive to antibiotic tested than *B. afzelii* strains.
6. The antibiotics take a long time to become effective.
7. The different killing kinetics of *B. burgdorferi sensu lato* strains can be of importance in a treatment regimen.

Persistence of *Borrelia burgdorferi s.l.* in Patients after Antibiotic Treatment

Patients and Methods

Patients: Patients were examined between October 1992 and August 1994. Clinical data are listed in Table 1.

Serological tests: Antibodies to *B. burgdorferi s.l.* in blood and CSF were determined by indirect immunofluorescence test (IFT) and ELISA as previously described [14].

Bacteriological examination: Mitral valve excisate, joint and skin biopsies were examined for *Borrelia* by darkfield microscopy and by culture in MKP medium as previously described [13, 15]. The cultures were incubated at 33°C for 5–15 weeks.

Isolates were identified with monoclonal antibodies L321F1 and L221F8 by Western blot [16] and by pulsed-field gel electrophoresis (PFGE) as described by *Casjens* and *Huang* [17] and *Busch* et al. [18].

Susceptibility of *B. burgdorferi s.l.* isolates to doxycycline, cefotaxime and ceftriaxone was evaluated with MIC_{90} in the MKP medium. The suitability of MKP medium for *in vitro* antimicrobial susceptibility testing for *B. burgdorferi s.l.* has been established previously [7, 8].

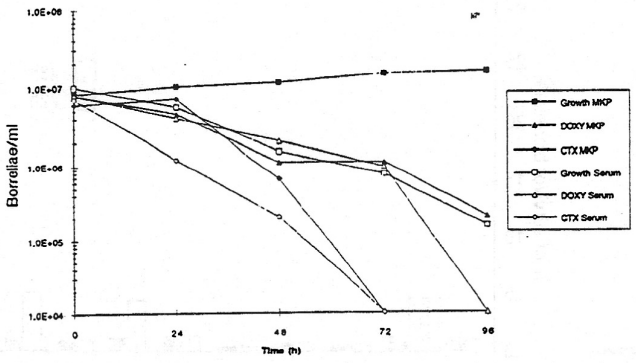

Figure 11: Kill kinetics: doxycycline (2 mg/l); cefotaxime (2 mg/l); strain PBre.

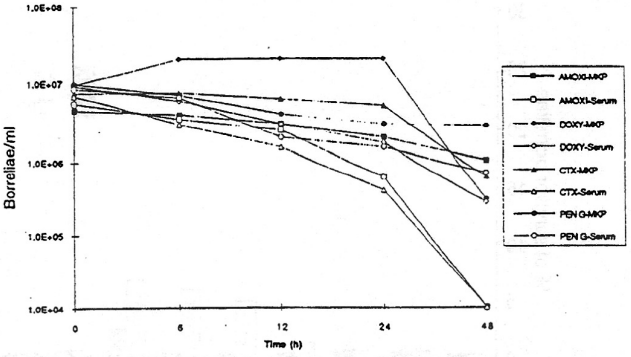

Figure 12: Kill kinetics: amoxicillin (4 mg/l); doxycycline (4 mg/l); cefotaxime (1 mg/l); penicillin G (8 mg/l); strain PKa.

Results

Borrelia isolation was successful in all cases after prolonged incubation of cultures, between 5–15 weeks in MKP medium. Using MAB L321F11 and L221F8 the isolates were grouped according to serotype [1, 2, 3, 4, 6, 7], with PFGE they were divided into genospecies *B. afzelii* and *B. garinii* (see cases).

Case 1

A 51-year-old man was admitted to the hospital in the autumn of 1987 because of plexus neuritis. The inflammatory parameters CAP and ESR were increased; the IgG antibody titers against *B. burgdorferi s.l.* in serum were positive (1:256), while the IgM titer was negative, Western blot showed typical *B. burgdorferi s.l.* bands. The patient was treated with cefotaxime 3x2 g per day i.v. for 12 days. In February 1988 the effect of the antibiotic therapy was controlled, antibody titers to *B. burgdorferi s.l.* and inflam-

Table 1: Patients with survival of *Borrelia burgdorferi s.l.* after antibiotic therapy. Clinical and microbiological findings.

Patient / Diagnosis		Treatment	Antibodies to *B. burgdorferi s.l.* Serum IgM	Serum IgG	Isolation of *B. burgdorferi s.l.*
1.	Plexus neuritis/ LMR-Bannwarth (1987)	Cefotaxime (6 g/day – 12 days)	NEG	POS	ND
	7 years cardiopathy	-----			
	Progressive cardiac symptoms (1993)	Mitral valve replacement	NEG	NEG	Mitral valve biopsy
2.	Painful knee arthritis	Ibuprofen Ceftriaxone (2 g/day – 14 days)	POS	POS	Synovial biopsy
3.	Painful knee arthritis	Corticosteroid Ceftriaxone (2 g/day – 14 days)	NEG	POS	Joint fluid
4.	Arthralgias/suspected Lymphocytoma benignum (1-year history)	Ceftriaxone (2 g/day – 14 days)	NEG	NEG	Skin biopsy (non-motile) (after subcultivation conversion to motile)
	After 7 months persistent arthralgias	Doxycycline (200 mg/day – 10 days)	NEG	NEG	Skin biopsy (non-motile)
5.	Arthralgias (knee, hand, shoulder)	Corticosteroid Doxycycline (200 mg/day – 10 days)	NEG	NEG	ND
	1 $^1/_2$-year history of arthralgias		NEG	NEG	Synovial hand biopsy

NEG = negative; POS = positive; ND = not done.

matory parameters were negative. In January 1993, the patient had a new attack. He claimed to have had a headache, attacks of perspiration and pseudoradicular pain located in the region of the right arm plexus. The antibody titers against *B. burgdorferi s.l.* and Western blot were negative. A tick bite in the summer of 1992 is dubious. Erythema migrans was not seen. In September 1993, the patient had a relapse with progressive cardiac pain and dyspnoea on exertion. Angiography and echocardiography revealed a 3rd degree mitral insufficiency. Furthermore, a break of tendon filament of the papillary muscle was diagnosed. The patient had a history of 7 years of cardiopathy. In November 1993, he was admitted to a university hospital where a replacement of the mitral valve was carried out. Intraoperatively, a heavy degenerative alteration of an already primary dysplastic mitral valve with defective substance and multiple tendon filament breaks on the anterior and posterior valve velum were found. The mitral valve excisate was investigated for culture isolation of borreliae due to his Lyme history (1987 Lyme IgG +, WB +). *Borrelia* was cultured from the excisate after prolonged incubation (9 weeks) in MKP medium. IgM and IgG antibody titers against *B. burgdorferi s.l.* (ELISA, IFT, Immunoblot) were negative. The patient was then treated with ceftriaxone.

Case 2

A 13-year-old otherwise healthy boy was admitted to a department of infectious disease because of intensive pain and swelling in the right knee. He had a 1-year history of attacks of pain. A tick bite was not remembered, an erythema migrans had never been seen. On admission, cultures of the effusion revealed no growth of bacteria but increased *B. burgdorferi s.l.* specific IgG and IgM (1:512 w.o. absorption) levels in serum. After puncture he developed fever and swelling also in the left knee.

The patient received ceftriaxone 2 g/day for 14 days. The swelling decreased but several days after treatment, the swelling of the right knee and right ankle recurred; there was no pain. At that point ibuprofen was given. The knee swelling continued for 4 months, serum Lyme IgG was 1:256 (w.o. absorption). The patient received ibuprofen for several weeks. During a 4-month period, the intensity of his symptoms always increased after a longer walk. When he was readmitted, 6 months after the first admission, a severe swelling of the right knee, limited mobility and a hydarthrosis were diagnosed. The symptoms in the left knee were less intensive. Complete hospital records including all clinical and laboratory investigations were obtained. The biopsy samples from the synovia and the effusion were examined for mycobacteria and *Borrelia*. There was no serological evidence of a bacterial or viral infection except that of Lyme borreliosis (IgG 1:128 w.o. absorption). *B. afzelii* was cultured from the synovia as well as from the effusion. At the follow-up examination, 1 month after the synovectomy and aspiration, the swelling and the effusion in the right knee were diagnosed. After a course of 14 days of ceftriaxone the swelling and effusion disappeared.

A second synovia examination, 6 months after the first synovectomy, showed an exudative synovitis. Lyme serology and *B. burgdorferi s.l.* cultures were negative. Within a few weeks of conservative therapy, the synovitis was cured, whereas the limitation of flexibility persisted. The last follow-up examination in 1993 confirms this finding.

Case 3

A 17-year-old man developed recurrent episodes of a painful knee and was treated with a corticosteroid. In early 1993, he was admitted to a department of rheumatology because of painfu swelling in the left and right knees. The physical and laborator examinations indicated arthritis in both knees as well as in the right elbow. Upon physical examination the patient was febrile Serological examinations for rheumatoid factor and syphili were negative, whereas serum Lyme IFT-IgG was borderline The patient was treated with ceftriaxone 2 g/day for 14 days.

In October 1993, he was suffering from recurrent arthritis in both knees. The IgG antibody titers against *B. burgdorferi s.l.* in the serum and in synovial fluid were positive (IFT 1:512, ELISA > 24) and IgM was negative.

B. afzelii was isolated from the effusion after 5 weeks of incubation in MKP medium. Subsequently cefotaxime was given 6 g/day for 14 days. Erythema migrans was not noted, although a tick bite was recalled. Antibiotic treatment resulted in marked reduction of complaints.

Case 4

A 35-year-old man was admitted to a dermatologic clinic with a 1-year history of headaches, intensive back pain, skin eruption and arthralgias. On admission, a lymphocytoma benignum was suspected. The neurological examination was normal. Serum Lyme IgG and IgM were negative. However, non motile borreliae were isolated from skin biopsy. After four subcultures in MKP medium enriched with 10 p.c. of a 35 p.c. albumin bovine solution (Sigma, Germany), the borreliae were motile. With MAB and PFGE, the strains were classified as *B. burgdorferi* serogroup 2 and genospecies *B. garinii*. A tick bite was not noted. The patient was treated with ceftriaxone 2 g/day for 14 days. The back pain gradually diminished, whereas the other symptoms persisted. The patient received doxycycline for 10 days in February 1993. In March 1993 the patient was readmitted due to persistent arthralgias. Antibody titers against *B. burgdorferi s.l.* in serum were negative, but *Borrelia* was isolated from a subsequent biopsy (taken in the immediate vicinity of the prior biopsy). This isolate remained impassive and oral penicillin G was given for 14 days. At a follow-up examination in June 1993, *B. burgdorferi s.l.* serology and cultures were negative. At the last examination in December 1993 the Lyme serology was negative, and culture was not done. The antibiotic treatment resulted in a reduction of arthralgias.

Case 5

A 28-year-old woman developed recurrent episodes of pain in her knee, hand. shoulder and of the talus and was treated with corticosteroids and doxycycline. After a 2-year history of pain and an increase of inflammation in the knee and hand joints a synovectomy was performed. Culture from biopsies for borreliae and Lyme serological tests were done. Lyme IgM and IgG were negative, nevertheless *B. afzelii* was isolated from hand synovia and the patient was treated with ceftriaxone.

Discussion

The main problems of Lyme disease are the lack of reliable diagnostic tests for actual disease plus the fact that the therapy can often be unsuccessful. The interpretation of serological tests and results may not be straightforward false-positive and false-negative results occur. Negative serological results do not necessarily exclude a *B. burgdorferi s.l.* infection [1–4]. Furthermore, it is unknown whether a positive PCR really indicates an active infection. Un-

fortunately, however, borreliae are easy to overlook in patients' samples, and not easy to detect. The overlapping symptomatology of the stages and the self-limited course of the infection in primary and secondary stages of the disease make diagnosis and therapy control more difficult.

According to *Asbrink* et al. [19] 10–15% of European patients with an untreated erythema migrans will develop neuroborreliosis. Months after the infection 60% of untreated American patients develop intermittent attacks of arthritis, 10% of the patients develop chronic Lyme arthritis. In 10% of the patients with arthritis the inflammation persists despite antibiotic treatment [20, 21]. However, the actual incidence of disseminated infection with manifestation in the heart and other organs is unknown. Furthermore, we have no data about the incidence of disseminated disease despite antibiotic therapy. How often borreliae may persist in the CSF, skin or other tissues, or their effect in producing atypical manifestations of the disease is not known. Our results in the isolation of *B. afzelii* from CSF during erythema migrans without inflammatory signs and neurological symptoms support very early dissemination [4]. The isolation of *B. burgdorferi sensu stricto (s. s.)* from iris biopsy, after several years of chronic recurrent uveitis and panuveitis with bilateral iridocyclitis despite antibiotic treatment, is the best evidence for long-time persistence of *Borrelia* apart from the CSF and skin [4]. Also in patients presented here, *Borrelia* survived months to years despite seemingly adequate-aggressive antibiotic treatment. These persisters are interesting because of the site of the persistence as well as the isolation after repeated antibiotic treatment. Especially interesting is the isolation of non-motile atypical forms of borreliae which after subcultures in the MKP medium become motile. This finding, as well as the results of other examinations [22], suggested the formation of spheroplast L-form variants in *B. burgdorferi s.l.* strains. According to experience with other bacteria, these forms can be responsible for chronic-persistent infection [23, 24].

The reason for the persistence of *B. burgdorferi s.l.* in patients after treatment with antibiotics is not completely understood. In fact a number of factors may play a role, e.g. virulence of borreliae, long generation time of borreliae, biologic differences in strains, the site of the infection, the ability of the drug to penetrate that site, insufficient antibiotic therapy and many others. To what extent the immunological status of a patient is of importance is unknown. The capacity of *Borrelia* to hide in various human tissues (heart, muscle, spleen, eyes, brain) [25–27, 4, 11, 28], intracellular localization and an insufficient antibiotic tissue level are critical for the therapy.

It is known that the therapy of late stages of Lyme disease can be complicated. Frequently, there are known recurrences of the manifestations and persistence of *Borrelia* despite seemingly adequate antibiotic treatment. The persistence and clinical recurrence in the erythema migrans stage are rarely noted, as the therapy seems to be mostly effective and adequate [3]. However, in most erythema migrans patients clinical and serological control of the effect of therapy, if any, is done a few months after the completion of antibiotic therapy, too short a period for the final diagnosis. Furthermore, proof of a successful therapy is based not only on the disappearance of clinical symptoms but also on the elimination of *Borrelia*; this is difficult to demonstrate and seldom done. The fact that antibodies against *B. burgdorferi s.l.* may persist for months, can indicate prior exposure to borreliae as well as active disease. Finally, we must take into consideration that changes in clinical symptomatology (after months; years) can lead the patients to change doctors.

The current antibiotic therapy (antibiotic, dosage, duration) of Lyme borreliosis varies greatly, therefore we have very different clinical and laboratory findings. Often the treatment seems to be wrong when ineffective antibiotics such as co-trimoxazole, cefaclor and aminoglycosides are given. According to the results of *in vitro* examinations and data of a few clinical studies, the cephalosporins are more efficient than penicillin G [29–31]. On the other hand some investigators [32–34] have reported no difference in the outcome between patients who received penicillin or those given cephalosporins. Furthermore, the comparison of penicillin G and oral doxycycline for treatment of neuroborreliosis [35] and azithromycin, doxycycline and phenoxymethylpenicillin for erythema migrans [36] show similar clinical efficacy. The CSF concentrations of penicillin, cefotaxime and ceftriaxone demonstrate that the cephalosporins penetrate to a greater extent than penicillin [33, 37]. The outcome of our studies on the kill kinetics and the killing effect show that the kinetics of killing borreliae differ from antibiotic to antibiotic. The killing rate of given antibiotics was dependent on the concentration and reaction time. However, killing *Borrelia* with β-lactams was more time dependent. The tested antibiotic first showed a significant killing effect between 48–72 h. The antibiotics were slowly effective. An interesting observation was the different effect of one antibiotic on various *B. burgdorferi s.l.* isolates in the MKP medium as well as in serum of healthy volunteers. Furthermore, the results suggest that the strains of *B. afzelii* and *B. garinii* have a different susceptibility to antibiotics used in the treatment of Lyme borreliosis. This individual action time of antibiotics in strains can be of importance in the treatment regimen. It is usually recommended that patients with erythema migrans be treated for at least 14 days with oral antibiotics (doxycycline 2x100 g/day, amoxicillin 2x400 mg/day and in complicated or late disease for 2–3 weeks with a systemic (cefotaxime 3x2 g/day, ceftriaxone 1x2 g/day). However, with the unpredictable progression of the disease and early dissemination of the borreliae, it seems appropriate to treat patients in stage 1 as effectively as possible. Complicated as well as late disease begin with early manifestation. Furthermore, the results of randomized prospective therapy studies and case reports show that with recommended antibiotic therapy cure is often impossible with only one treatment course.

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Therefore, for the potentiation of treatment the intensification of antibiotic therapy by establishing a new dosage regimen can be of great importance. A combination of two antibiotics (cephalosporin/doxycycline, cephalosporin/azithromycin, penicillin G/in combination) must be taken into consideration. Likewise a pulsed (interval) therapy with larger doses of antibiotics for 4–5 days, twice with an antibiotic free interval of 4–5 days, seems to be advantageous. This treatment regimen, which takes into consideration the long generation time of *Borrelia* and the antibiotic mechanism of action, can probably be more effective than the regimen used. The higher doses of antibiotics reach correspondingly effective higher serum, CSF and tissue antibiotic concentrations and repeated doses of antimicrobial can kill the survivor borreliae as effectively as the initial dose. Furthermore, it is known that the bacteria in the post-antibiotic effect phase of growth are more susceptible to the antibacterial activity of human leukocytes than untreated bacteria.

In conclusion, the isolation of *B. burgdorferi s.l.* after antibiotic treatment as well as the persistence of clinical signs demonstrate that currently recommended treatment regimens are inadequate for some patients. Because of this observation it has become questionable whether a definite eradication of borreliae with one antibiotic course is always possible. The therapy ought to be realized and controlled more on an individual basis. All patients respond differently. We consider the optimal therapy for the erythema migrans stage to be very important in early blocking of the dissemination of *Borrelia*. The association of *B. burgdorferi s.s.*, *B. garinii* and *B. afzelii* with different clinical manifestation of Lyme disease we can not confirm (paper in preparation), but we have found differences in the killing effect of various antibiotics on strains of these three species. Perhaps we have a correlation here. The questions of why a 100% eradication of borreliae is not possible, why a 100% resolving of clinical symptoms is not achieved if borreliae are susceptible to antibiotics used in treatment (treatment failure? other factors?) could be answered only by extensive and rigorously designed clinical and microbiological studies. Possibly, with persistence of disease or recurrence of clinical symptoms we have to consider atypical forms of *B. burgdorferi s.l.*, spheroplast L-form variants. The eradication of these forms as well as the intracellularly localized borreliae [38] is difficult and with β-lactam antibiotics practically not obtainable. Here combination therapy with doxycycline or macrolides is indicated.

Zusammenfassung: Abtötungskinetik der *Borrelia burgdorferi s.l.* – bakteriologische und klinische Befunde: Die bakterizide Aktivität von Amoxicillin, Doxycyclin, Cefotaxim, Ceftriaxon, Azithromycin und Penicillin G gegen *Borrelia burgdorferi s.l.* Stämme wurde in MKP-Medium und Humanserum während der 72 Stunden Einwirkungszeit untersucht. Die Antiborrelienwirkung der Antibiotika wurde an 20 Patientenisolaten, zehn *Borrelia afzelii* und zehn *Borrelia garinii* Stämmen, getestet. Der Abtötungseffekt der einzelnen Antibiotika auf getestete Stämme ist sehr unterschiedlich. Die Unterschiede hinsichtlich der Keimreduktion bestehen zwischen den Stämmen der *B. afzelii* und *B. garinii* species als auch zwischen den Stämmen innerhalb einer Spezies. Der Anteil der abgetöteten Borrelien ist weniger abhängig von der Konzentration des Antibiotikums als von der Einwirkungszeit. Die *B. garinii* Stämme sind offensichtlich empfindlicher gegen die in der Therapie eingesetzten Antibiotika. Die Persistenz von *B. burgdorferi s.l.* und Rezidive der Erkrankung nach der Antibiotikatherapie werden diskutiert.

References

1. **Pfister, H.-W., Preac Mursic, V., Wilske, B., Einhäupl, K. M., Weinberger, K.:** Latent Lyme neuroborreliosis: presence of *Borrelia burgdorferi* in the cerebrospinal fluid without concurrent inflammatory signs. Neurology 39 (1989) 1118–1120.
2. **Preac Mursic, V., Weber, K., Pfister, H.-W., Wilske, B., Gross, B., Baumann, A., Prokop, J.:** Survival of *Borrelia burgdorferi* in antibiotically treated patients with Lyme borreliosis. Infection 17 (1989) 355–359.
3. **Liegner, K., Shapiro, J., Ramsay, D. R., Halperin, A. J., Hagrefe, W., Kong, L.:** Recurrent erythema migrans despite extended antibiotic treatment with minocycline in a patient with persisting *Borrelia burgdorferi* infection. J. Am. Acad. Dermatol. 28 (1993) 312–314.
4. **Preac Mursic, V., Pfister, H.-W., Spiegel, H., Burk, R., Wilske, B., Reinhardt, S., Boehmer, R.:** First isolation of *Borrelia burgdorferi* from an iris biopsy. J. Clin. Neuroophthalmol. 13 (1993) 155–161.
5. **Johnson, R. C., Kodnes, C., Russel, M.:** *In vitro* and *in vivo* susceptibility of the Lyme disease spirochete *Borrelia burgdorferi* to four antimicrobials. Antimicrob. Agents Chemother. 31 (1987) 164–167.
6. **Johnson, R. C.:** Isolation techniques for spirochetes and their sensitivity to antibiotics *in vitro* and *in vivo*. Rev. Infect. Dis. 11 (Suppl. 6) (1989) 1505–1510.
7. **Preac Mursic, V., Wilske, B., Schierz, G., Holmburger, M., Süß, E.:** *In vitro* and *in vivo* susceptibility of *Borrelia burgdorferi*. Eur. J. Clin. Microbiol. 6 (1987) 424–426.
8. **Preac Mursic, V.:** Antibiotic susceptibility of *Borrelia burgdorferi. In vitro* and *in vivo*. In: *Weber, K., Burgdorfer, W.* (eds.): Aspects of Lyme borreliosis. Springer, Berlin, Heidelberg, New York 1993, pp. 301–311.
9. **Levin, J. M., Nelson, J. A., Segreti, J., Harrison, B., Benson, C. A., Strle, F.:** *In vitro* susceptibility of *Borrelia burgdorferi* to 11 antimicrobial agents. Antimicrob. Agents Chemother. 37 (1993) 1444–1446.
10. **Schmidli, J., Hunziker, T., Moesli, P., Schaad, U. B.:** Cultivation of *Borrelia burgdorferi* from joint fluid three months after treatment of facial palsy due to Lyme borreliosis. J. Infect. Dis. 158 (1988) 905–906.
11. **Häupl, T., Hahn, G., Rittig, M., Krause, A., Schoerner, C., Schönherr, U., Kalden, J. R., Burmester, G. R.:** Persistence of *Borrelia burgdorferi* in ligamentous tissue from a patient with chronic Lyme borreliosis. Arthritis Rheum. 36 (1993) 1621–1626.
12. **Liegner, K. B.:** Treatment of late Lyme disease: a challenge to accept. J. Clin. Microbiol. 32 (1994) 1415–1416.
13. **Preac Mursic, V., Wilske, B., Schierz, G.:** European *Borrelia burgdorferi* isolated from humans and ticks: culture conditions and antibiotic susceptibility. Zbl. Bakt. Hyg. A 263 (1986) 112–118.
14. **Wilske, B., Preac-Mursic, V.:** Microbiological diagnosis of Lyme bor-

reliosis. In: *Weber, K., Burgdorfer, W.* (eds.): Aspects of Lyme borreliosis. Springer, Berlin, Heidelberg, New York 1993, pp. 267–300.
15. **Preac Mursic, V., Wilske, B.:** Biology of *Borrelia burgdorferi*. In: *Weber, K., Burgdorfer, W.* (eds.): Aspects of Lyme borreliosis. Springer, Berlin, Heidelberg, New York 1993, pp. 44–58.
16. **Wilske, B., Preac Mursic, V., Fuchs, R., Bruckbauer, H., Hofmann, A., Zumstein, G., Jauris, S., Soutschek, E., Motz, M.:** Immunodominant proteins of *Borrelia burgdorferi*; implications for improving serodiagnosis of Lyme borreliosis. In: *Neu, H. C.* (ed.): New antibacterial strategies. Churchill Livingstone, London 1990, pp. 47–63.
17. **Casjens, S., Huang, W. M.:** Linear chromosomal physical and genetic map of *Borrelia burgdorferi*, the Lyme disease agent. Molec. Microbiol. 8 (1993) 967–980.
18. **Busch, U., Teufel, C. H., Boehmer, R., Wilske, B., Preac-Mursic, V.:** Molecular characterization of *Borrelia burgdorferi sensu lato* strains by pulsed-field gel electrophoresis. Electrophoresis 16 (1995) 744–747.
19. **Asbrink, E., Olsson, I., Hovmark, A.:** Erythema chronicum migrans afzelius in Sweden. A study on 231 patients. Zbl. Bakt. Hyg. A 263 (1986) 229–236.
20. **Steere, A. C., Schoen, R. T., Taylor, E.:** The clinical evolution of Lyme arthritis. Ann. Intern. Med. 107 (1987) 725–731.
21. **Steere, A. C., Levin, R. E., Molloy, P. J., Kalish, R. A., Abraham III, J. H., Lui, N. Y., Schmid, C. H.:** Treatment of Lyme arthritis. Arthritis Rheum. 37 (1994) 878–888.
22. **Radolf, J. D., Bourell, K. W., Akins, D. R., Brusca, J. S., Norgard, M. V.:** Analysis of *Borrelia burgdorferi* membrane architecture by freeze-fracture electron microscopy. J. Bacteriol. 176 (1994) 21–31.
23. **Swain, R. H. A.:** Electron microscopic studies of the morphology of pathogenic spirochaetes. J. Path. Bact. Vol. LXIX (1955) 117–128.
24. **Charache, P.:** Atypical bacterial forms in human disease. In: *Guze, L. B.* (ed.): Microbial protoplasts, spheroplasts and L-forms. Williams and Wilkins, Baltimore 1968, p. 484.
25. **Stanek, G., Klein, J., Bittner, R., Glogar, D.:** Isolation of *Borrelia burgdorferi* from the myocardium of a patient with longstanding cardiomyopathy. N. Engl. J. Med. 322 (1990) 249–252.
26. **Detmar, U., Maciejewski, W.:** Borrelial dermatomyositis-like syndrome. In: *Weber, K., Burgdorfer, W.* (eds.): Aspects of Lyme borreliosis. Springer, Berlin, Heidelberg, New York 1993, pp. 259–266.
27. **Cimmino, M., Azzolini, A., Tobia, F., Pesce, C.:** Spirochetes in the spleen of a patient with chronic Lyme disease. Am. J. Clin. Pathol. 91 (1989) 95–97.
28. **MacDonald, A. B.:** *Borrelia* in the brains of patients dying with dementia. JAMA 256 (1986) 2195–2196.
29. **Dattwyler, R. J., Halperin, J. J., Volkman, D. J., Luft, B. J.:** Treatment of late Lyme borreliosis – randomised comparison of ceftriaxone and penicillin. Lancet ii (1988) 1191–1194.
30. **Pal, G. S., Baker, J. T., Wright, D. J. M.:** Penicillin-resistant borrelia encephalitis responding to cefotaxime. Lancet i (1988) 50–51.
31. **Hassler, D., Zöller, L., Haude, M., Hufnagel, H.-D., Heinrich, F., Sonntag, H.-G.:** Cefotaxime versus penicillin in the late stage of Lyme disease – prospective, randomized therapeutic study. Infection 18 (1990) 16–20.
32. **Kristoferitsch, W., Baumhackl, U., Sluga, E., Stanek, G., Zeller, K.:** High-dose penicillin in meningopolyneuritis Garin-Bujadoux-Bannwarth. Zbl. Bakt. Hyg. A 263 (1986) 357–364.
33. **Pfister, H.-W., Preac-Mursic, V., Einhäupl, K. M., Wilske, B.:** Cefotaxime versus penicillin G for acute neurological manifestations of Lyme borreliosis: a prospective randomized study. Arch. Neurol. 46 (1989) 1190–1194.
34. **Weber, K., Preac-Mursic, V., Wilske, B., Thurmayr, R., Neubert, U., Scherwitz, C.:** A randomized trial of ceftriaxone versus oral penicillin for treatment of early Lyme borreliosis. Infection 18 (1990) 91–96.
35. **Karlsson, M., Hammers-Berggren, S., Lindquist, L., Stiernstedt, G., Svenungsson, B.:** Comparison of intravenous penicillin G and oral doxycycline for treatment of Lyme neuroborreliosis. Neurology 44 (1994) 1203–1207.
36. **Strle, F., Stanek, G., Ruzic, E., Michieli, M. S.:** Erythema migrans: comparison of treatment with azithromycin, doxycycline and phenoxymethylpenicillin. Congress for Infectious Diseases, Montreal, Canada, 1990.
37. **Pfister, H.-W., Preac Mursic, V., Wilske, B., Schielke, E., Soergel, F., Einhäupl, K. M.:** Randomized comparison of ceftriaxone and cefotaxime in Lyme neuroborreliosis. J. Infect. Dis. 163 (1991) 311–318.
38. **Ying, M., Sturrock, A., Weis, J. J.:** Intracellular localization of *Borrelia burgdorferi* within human emdothelial cells. Infect. Immun. 59 (1991) 671–678.

Infection 24 (1996) No. 1 © MMV Medizin Verlag GmbH München, München 1996