Active Infection: Clinical Definitions and Evidence of Persistence in Lyme Disease—Contesting the Underlying Basis for Treatment Limitations for Early and Late Lyme Disease, as well as Chronic Lyme Disease, Alternatively Known as "Post-Lyme Disease Syndrome"

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This Challenge is to the Following Contested Recommendation:

> *"To date, there is no convincing biologic evidence for the existence of symptomatic chronic B. burgdorferi infection among patients after receipt of recommended treatment regimens for Lyme disease. Antibiotic therapy has not proven to be useful and is not recommended for patients with chronic (6 months) subjective symptoms after administration of recommended treatment regimens for Lyme disease (E-I)."*
>
> (IDSA Guidelines for Lyme Disease, pp. 1120-21)

I. Introduction

Clinical Descriptions

The IDSA Guidelines (The Guidelines), attempt to define the language describing patients with persistent or recurrent subjective symptoms of Lyme disease despite a 30 day course of recommended antibiotic therapy. Such patients are difficult to clinically categorize, largely due to an expansive array of complaints which, although typically lacking the objective findings described by CDC surveillance case definition, may include other objective features.

The Guidelines characterize these patients as having "post–Lyme disease syndrome", "posttreatment chronic Lyme disease", or "chronic Lyme disease" and use these terms synonymously. For the sake of simplicity, in this analysis such patients will most commonly be referred to as having chronic Lyme disease. The Guidelines also suggest that such patients must have been previously diagnosed with a case of Lyme disease which had met CDC surveillance case definition.

The Guidelines further endeavor to distinguish chronic Lyme disease from "late Lyme disease" as they pertain to the presence or absence of the objective findings described by CDC surveillance case definition. The Guidelines use this case definition to describe active late Lyme disease, which it recommends should be re-treated with antibiotics, but only if relapse is demonstrated by objective measures. Patients with persistent subjective symptoms of active late Lyme disease, either absent objective features described by CDC surveillance case definition, or inclusive of objective features not described by this case definition, are defaulted into a post-infectious syndrome.

Use of CDC surveillance case definition in this manner is contradictory to a directive by the CDC, which states, "This surveillance case definition was developed for national reporting of Lyme disease; it is not intended to be used in clinical diagnosis.[1]" The CDC's admonition against employing surveillance criteria for diagnosis is compatible with the findings that studies of both early and late Lyme disease demonstrate that in many to most cases, objective signs as described by CDC surveillance case definition are lacking. Consequently, confining diagnosis to surveillance criteria would result in the failure to diagnosis and treat those patients with active Lyme disease who do not fall within the surveillance definition.

II. Review of Evidence

Contested Recommendation:

"To date, there is no convincing biologic evidence for the existence of symptomatic chronic B. burgdorferi infection among patients after receipt of recommended treatment regimens for Lyme disease. Antibiotic therapy has not proven to be useful and is not recommended for patients with chronic (6 months) subjective symptoms after administration of recommended treatment regimens for Lyme disease (E-I)." (IDSA Guidelines for Lyme Disease, pp. 1120-21)

A. Evidence Cited by The Guidelines in Support of Recommendation—and Critique of that Evidence

In this section, the portions of The Guidelines which are germane to the contested recommendation will be reviewed. Claims and supporting evidence cited by The Guidelines will be critically evaluated as to quality and scientific accuracy. Counter-evidence will be presented where appropriate. The framework of The Guidelines will provide a partial structure to this section, as each claim is argued in sequence. **Throughout this analysis,** "⌘" **before an author's name identifies him/her as an author of The Guidelines.**

Biologic Plausibility

On page 1118, The Guidelines state, *"The notion that symptomatic, chronic B. burgdorferi infection can exist despite recommended treatment courses of antibiotics in the absence of objective clinical signs of disease, is highly implausible as evidenced by:"*

1. *"the lack of antibiotic resistance in this genus"*

B. burgdorferi antimicrobial resistance against all the major antibiotic classes used against Lyme disease, and even against those less well used, has been documented repeatedly by ⌘Wormser,[2] ⌘Strle,[3] and numerous other researchers.[4,5,6,7]

2. *"the lack of correlation of persistent symptoms with laboratory evidence of inflammation or with the eventual development of objective physical signs"*

First, The Guidelines do not evaluate the spectrum of laboratory evidence of inflammation that has been reported as the result of chronic *B. burgdorferi* infection. For example, late Lyme disease patients may be more likely to manifest inflammatory markers that are not routinely evaluated, such as interleukin-18 and interleukin-1beta,[8] as well as circulating

immune complexes.[25,155,167,168] Generally limited and/or inconsistent inflammation in late Lyme may be due in part to the well recognized anti-inflammatory effects of *B. burgdorferi*.[9,10] This is compatible with the finding that *B. burgdorferi* can cause disease in the absence of a histologically demonstrable inflammatory infiltrate.[52]

Second, when the presence of objective features described by CDC surveillance case definition is not required as a defining entrance criterion for studies of active late Lyme disease, then scrutiny for the development of "*objective physical signs*" can occur without selection bias. Rather, when direct detection of the organism by PCR or culture becomes the focus of study, it becomes clear that many to most patients with documented active late Lyme disease primarily manifest either objective signs that are not described by CDC surveillance case definition or subjective symptoms only.[39,47,54,150,161,167,177,186,211,212]

3. *"the lack of precedent for such a phenomenon in other spirochetal infections"*

Well recognized in *B. burgdorferi* infection,[10,11] *T. pallidum* also induces interleukin-10 production, which in turn contributes to its anti-inflammatory properties.[12] Other mechanisms of immune evasion and/or tolerance can be linked to poorly immunogenic outer surface proteins that are expressed by both *B. burgdorferi* and *T. pallidum*.[13]

On page 1118, The Guidelines authors claim what they term *"additional compelling evidence"*, stating, *"The panel is unaware of any chronic infection in which antibody titers diminish despite persistence of the causative organism."*

There are many examples of chronic infections with negative serologies, including syphilis,[14,15] amebiasis,[16] HIV,[17,18] and Hepatitis C, both with,[19] and without HIV co-infection.[20] However, rather than drawing comparisons to "*any chronic infection*", **truly** compelling evidence might actually come by evaluating the data on chronic Lyme disease itself in which antibody titers diminish despite documented persistence of *B. burgdorferi* after antibiotic failure. Such diminution of Lyme antibody titers despite persistent infection has already been demonstrated, not only in good animal studies,[127] but in abundant human trials as well.[39,47,167,170] None of this data has been referenced by The Guidelines.

On page 1101, The Guidelines state that in regard to patients with a clinically uncertain diagnosis of erythema migrans (EM), *"Untreated patients who remain seronegative, despite continuing symptoms for 6–8 weeks, are unlikely to have Lyme disease, and other potential diagnoses should be actively pursued."*

Given that the clinical presentations of EM can be heterogeneous, accurate diagnosis of this skin lesion by physicians has been inconsistent.[21,185] As such, even when EM is clinically uncertain, prudence dictates to err on the side of treatment without waiting for serologic evidence. Moreover, there is abundant data not referenced by The Guidelines that seronegative active Lyme disease with continuing symptoms for greater than 6-8 weeks not only exists, but that it is common. Although seronegative Lyme disease is not the focus of this portion of testimony by design, it is appropriate to provide brief comment for the record.

For example, in a study of 41 patients with positive culture and/or PCR proven active late Lyme disease, 63.5% did not have fully reactive Lyme serologies despite that 54% had been symptomatic for over a year.[22] The authors state, "We conclude that antibodies to *B. burgdorferi* often are present in only low levels or are even absent in culture or PCR positive patients who have been suffering for years from symptoms compatible with LB [Lyme borreliosis].[22]"

In a second study of 32 patients hospitalized for late Lyme, whose disease activity was confirmed by positive PCR, 56.3% were seronegative,[23] prompting the authors to state, "In the seronegative patients with Lyme borreliosis symptoms, additional testing should be introduced."

In a third study of 10 patients with late ocular Lyme disease, 50% did not have fully positive Lyme serologies,[24] the authors concluding, "Late-phase ocular Lyme borreliosis is probably underdiagnosed because of weak seropositivity or seronegativity in ELISA assays.[24]"

In addition to the previous three published studies, many others demonstrate that seronegative late active Lyme disease is common, including but not limited to 49 more as referenced.[186,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,161 , 167,170]

On page 1101, The Guidelines further state, "*For patients with symptoms in excess of 4 weeks to be considered seropositive, reactivity must be present on the IgG immunoblot specifically*."

Yet ⌘Steere has established repeatedly that persistent IgM late into the illness has meaning. Even early into the study of Lyme disease, before the spirochetal etiology was discovered and specific serologies were in use, in prospectively followed patients both total serum IgM and

cryoglobulins were increased during periods of disease exacerbation.[69] In later studies using specific Lyme serologies, there were similar findings. For example, while studying 48 patients with various stages of Lyme disease, ⌘Steere found that, "… serum IgM levels correlated directly with disease activity (p = 0.025)… [70]"

In a later study of 46 children with Lyme arthritis who went untreated for at least 4 years, ⌘Steere found, "…the children with recurrent symptoms more often had IgM responses to the spirochete…[71]"

In another study, in regard to the persistence of IgM Lyme serologies in late disease, ⌘Steere wrote, "…it may persist in later disease…" and "…its persistence implies ongoing antigenic stimulation of the immune system, perhaps by an intact spirochete" and "…persistence of specific IgM antibodies may also be associated with more severe disease.[72]" All of these conclusions suggest significance to the findings of persistent Lyme IgM reactivity in late Lyme disease. Of significance, false positive Lyme IgM ELISA results were not demonstrated.[72]

Others have corroborated his findings, demonstrating persistent Lyme IgM serologic reactivity during periods of Lyme PCR positivity, and IgM seroreversion in association with Lyme PCR negativity; whereas Lyme IgG serologic status did not correlate Lyme PCR status.[166] Given the foregoing, persistent reactivity of Lyme IgM antibodies most likely has value in the assessment of Lyme disease patients whose illness extends beyond 4 weeks duration.

On page 1118, The Guidelines state that *"Lyme disease lacks characteristics of other infections that justify longer treatment",* such as:

1. *"infections in immunodeficient hosts"*

B. burgdorferi infection establishes immunosuppression in the host by multiple mechanisms.[9,10,73,74] This results in significant immune tolerance which in turn permits chronic infection. These host interactions were not evaluated by The Guidelines.

2. *"infections caused by an intracellular pathogen"*

There is robust data that *B. burgdorferi* establishes intracellular infection.[52,75,76,77,78,79,80] Thirteen years before the publication of The Guidelines, ⌘Klempner verified not only the presence of *B. burgdorferi* intracellular infection within fibroblasts and keratinocytes, but

that this intracellular sanctuary protects *B. burgdorferi* from certain antibiotics. ⌘Klempner states, "In these experiments, we demonstrated that fibroblasts and keratinocytes were able to protect *B. burgdorferi* from the action of this B-lactam antibiotic [ceftriaxone] even at antibiotic concentrations > or = 10 times the MBC of the antibiotic.[81]" Other authors have similarly demonstrated protection of *B. burgdorferi* by eukaryotic cells,[82] as acknowledged by ⌘Bockenstedt.[116] A protective action such as this is more likely to occur if *B. burgdorferi* has limited cytopathic effects on these cells.

Despite that multiple eukaryotic cells have been shown to maintain integrity[79] and thus protect intracellular *B. burgdorferi* against antibiotics,[81,82] there is data from one *in vitro* study which suggests that significant stress to invaded fibroblasts argues against intracellular persistence of *B. burgdorferi*.[83]

However, in another *in vitro* study by Livengood of the CDC,[79] when *B. burgdorferi* was evaluated for its ability to invade human neuroglial and cortical neuronal cells, the authors documented intracellular localization of *B. burgdorferi* in all neural cells tested. Most importantly, they state, "Cytopathic effects were not observed following infection of these cell lines with *B. burgdorferi*, and internalized spirochetes were found to be viable. Invasion of neural cells by *B. burgdorferi* provides a putative mechanism for the organism to avoid the host's immune response while potentially causing functional damage to neural cells during infection of the CNS.[79]"

Moreover, *in vivo* data demonstrates *B. burgdorferi* intracellular persistence in four additional studies as follows: First, synovial membrane biopsies from Lyme arthritis patients demonstrated both intact "Borrelia-like structures" and specific outer surface protein A intracellularly.[84] Second, in a study where *B. burgdorferi* was cultured from a chronic Lyme disease patient after failure of antibiotic therapy, the biopsy demonstrated "…numerous fibroblasts deeply invaginated by the spirochetes…[170]" In a third study, the authors found *B. burgdorferi* surviving within macrophages and keratinocytes from skin biopsies.[52] In a fourth study, the authors found *B. burgdorferi* intracellularly in the brains of 3 patients with neuroborreliosis.[80] In this last study, there was evidence of nuclear fragmentation, suggesting that this may lead to apoptosis. However, since *B. burgdorferi* was observed intracellularly from these *in vivo* experiments, cytopathic effects were limited.

As such, the preponderance of data indicates that: *B. burgdorferi* establishes intracellular infection within eukaryotic cells both *in vitro* and *in vivo*; intracellular infection results in limited, if any, cytopathic effects; and intracellular localization protects *B. burgdorferi* from certain antibiotics, including ceftriaxone.

On Page 1118, The Guidelines state, *"The "cystic" forms of B. burgdorferi that have been seen under certain growth conditions in vitro have not been shown to have any clinical significance."*

In support of this assertion, The Guidelines authors reference a single article by Alban.[85] However the reference does not agree with the IDSA's claim. In regard to the cystic forms, Alban refutes the IDSA's contention, instead stating, "they may represent a strategy that facilitates the survival of *B. burgdorferi*.[85]"

Other scientists have researched these forms in some depth, stating, "The results indicate that atypical extra- and intracellular pleomorphic and cystic forms of *Borrelia burgdorferi* and local neuroinflammation occur in the brain in chronic Lyme neuroborreliosis. The persistence of these more resistant spirochete forms, and their intracellular location in neurons and glial cells, may explain the long latent stage and persistence of Borrelia infection.[80]"

Many researchers have documented *B. burgdorferi* spheroplasts, of which both cystic forms and granules are sub-types, both *in vitro*[52,60,80,86,87,88,89,90,91,92] and *in vivo*.[60,80,93,94,95,96] Several authors have documented that these forms can revert back to spiral forms if environmental conditions are favorable.[65,97,98]

In vivo evidence demonstrates that these forms are virulent. For example, inoculation of mice with *B. burgdorferi* cystic forms results in infection with the subsequent recovery of spiral forms, which had reverted *in vivo,* from these infected animals.[99] In a human study evaluating both *in vitro* and *in vivo* mechanisms of *B. burgdorferi* pleomorphism, spiral forms of *B. burgdorferi* were cultured from the brain biopsies of neuroborreliosis patients in whom pleomorphic or cystic forms of *B. burgdorferi* were demonstrated. These cultured organisms were then able to infect neurons *in vitro*, the authors concluding, "…these surviving cultivatable spirochetes are still virulent.[80]"

This data has led several researchers to ascertain that *B. burgdorferi* cystic forms are materially responsible for the chronic and relapsing nature of Lyme disease.[80,100,101,208] There importance has been stressed, as researchers state, "The identification of these extra- or intracellular atypical, cystic and granular forms of *Borrelia burgdorferi* is essential for the histopathological diagnosis of Lyme disease as they may indicate chronic Borrelia infection, even in cases where the typical coiled spirochetes are apparently absent.[80]"

Variable outer surface protein expression, altered antibiotic resistance profiles,

ultrastructural DNA sequestration, dissimilar growth kinetics, and divergent culture media requirements compared to *B. burgdorferi* spiral forms, all help *B. burgdorferi* cystic, as well as other pleomorphic forms, to explain the observations of seronegativity in late disease, chronic infection despite antibiotic therapy, limited *in vivo* PCR sensitivity, and poor culture yields from patients with late Lyme disease when using standard media preferred for the cultivation of spiral forms, respectively.[52,80,96,98,102,103]

In sum, The Guidelines statement that cystic forms "…*have not been shown to have any clinical significance*" is not correct. Rather, these host adapted forms are critically important to improved diagnostics and the greater understanding of how *B. burgdorferi* causes disease in the human host.

Claims of "Overdiagnosis"

On page 1117, The Guidelines state, *"When adult and pediatric patients regarded as having chronic Lyme disease have been carefully reevaluated at university-based medical centers, consistently, the majority of patients have had no convincing evidence of ever having had Lyme disease, on the basis of the absence of objective clinical, microbiologic, or serologic evidence of past or present B. burgdorferi infection."* [253, 268, 295–298][104,105,106,107,108,109]

For most of these studies, the diagnoses of Lyme disease were re-evaluated on the basis whether or not they met CDC surveillance case definition. According to Paul Mead, M.D. M.P.H. of the CDC, surveillance case definitions "err on the side of specificity."[110] As such, diagnosing active Lyme disease by strict adherence to CDC surveillance case definition and erring on the side of specificity would result in underdiagnosis. Mead also states, "A clinical diagnosis is made for the purpose of treating an individual patient and should consider the many details associated with that patient's illness. Surveillance case definitions are created for the purpose of standardization, not patient care…[110]" Despite this, in the articles referenced by The Guidelines,[104,105,106,107,108,109] active Lyme disease is refuted mostly by failure to meet both clinical and laboratory aspects of CDC surveillance case definition.

Moreover, in the articles cited by The Guidelines[104,105,106,107,108,109] laboratory testing was handled as follows: If patients had prior positive Lyme serologies at outside institutions and were then found to be negative at the authors' institutions, then the authors deemed those first Lyme serologies to have been false positives.

For example, Reid expressed the opinion that 60% of evaluated patients never had any past or current evidence of Lyme disease, despite the fact that "Sixty-one percent had previously had a positive test result for Lyme disease…[104]"

Likewise, of the group of patients which ⌘Steere asserts did not have Lyme and were seronegative at his institution, 45% had previously had a positive Lyme serologic test result elsewhere.[106]

Rose found that in the "non-Lyme group" comprised of patients with only subjective symptoms of Lyme disease, 50% had partial to full seroreactivity against *B. burgdorferi* at prior laboratories, but Rose asserted none to be positive by his own testing.[107]

In an article by Sigal, 68% of the largest "not-Lyme" patient group, which the author had diagnosed with fibromyalgia, had prior positive serologies at other laboratories.[108] The repeated assumptions of superior Lyme serologies at the various authors' institutions as cited above may not be accurate.

Rather, there is evidence by ⌘Dattwyler and ⌘Steere, the latter also having been author of one of the "overdiagnosis" articles,[106] that some "university based medical centers" inclusive of their own and that of Sigal, author of two of the "overdiagnosis" articles,[105,108] use Lyme serologies that lack sensitivity.[111]

⌘Dattwyler and ⌘Steere evaluated Lyme serologies for the following 5 academic Lyme disease research centers: The Marshfield Clinic; University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School; State University of New York at Stony Brook; Tufts/New England Medical Center; and the University of Connecticut Health Center. Sensitivities ranged from extremely poor to inadequate as follows: 40%, 49%, 73%, 76%, 79%.[111] Identities of each testing center linking them individually to the sensitivity of their own test were not divulged, but it makes little difference as the best performer of the group still lacked adequate sensitivity. These university based facilities performed Lyme serologies in 3 out of the 6 articles alleging overdiagnosis,[105,106,108] providing additional basis for inaccurate conclusions.

In the fourth of the six alleged overdiagnosis articles, Yale University-Medical Center performed the Lyme serologies.[104] This university based medical center may also be using Lyme serologies which lack adequate sensitivity, evidenced as follows: A 24 year old woman, who had positive Lyme serologies at both the CDC and the New York State Department of Health but negative Lyme serologies at Yale, gave birth to a stillborn infant in whom fetal autopsy demonstrated *B. burgdorferi* in the liver, adrenal, brain, heart and placenta.[112] In

an unrelated but reminiscent second study, Lavoie wrote, "We report a culture positive neonatal death occurring in California, a low endemic region. ...Bb [*B. burgdorferi*] was grown from a frontal cerebral cortex..." The mother had been having migratory arthralgias, malaise, and other subjective symptoms but was seronegative at Yale.[113]

In the 2nd of the "overdiagnosis" articles by Sigal,[105] there is explicit circular reasoning and selection bias as follows: In order to arrive at the study population, Sigal "…reviewed the records of all patients younger than 20 years of age seen at The Lyme Disease Center at Robert Wood Johnson Medical School…in whom fibromyalgia had been diagnosed, with or without an antecedent diagnosis of LD [Lyme disease]." He states "we found 30 patients younger than the age of 20 who had fibromyalgia but no evidence of active LD." The diagnosis of fibromyalgia is both part of the entrance criteria and part of the conclusion, causing selection bias.

Overt circular reasoning is evident as Sigal states, "active LD was defined as having the clinical features ascribed to LD present at the time of our evaluation without the patient's having received adequate antibiotic therapy for that clinical problem." So by Sigal's *very definition*, anyone who had been treated with antibiotics for Lyme disease failed to meet the criteria for active Lyme. Not surprisingly he concluded, "None of the patients had active LD at the time of evaluation." This came despite the observation that "nearly one third of the courses of antibiotics did result in transient improvement of the complaints..."

As a potential source of the patients' recurrent and continued illness, Sigal theorized, "Pain and anxiety, on their own, can lead to sleep loss; this then causes more pain, establishing an unbroken cycle." This conclusion came despite the fact that he observed, "Rather than noting sleep disorder, many of the patients and parents stated that the patient slept TOO WELL,[capitalized in the text by Sigal]..." In sum, the article by Sigal is multiply flawed, but chiefly for reasons of selection bias, circular reasoning, and lapses of logic.[105]

In another of the "overdiagnosis" articles referenced by The Guidelines, Burdge wrote, "The most common scenario was for patients to have a skin lesion that they or their doctor thought might be EM."[109] He found, "74% of patients in this study had a history of skin rash." However, Burdge and colleagues incorrectly limited the definition of EM to include only those rashes with central clearing, attempting to retrospectively refute the prior EM diagnoses, for rashes that the authors did not themselves witness. ⌘Steere has demonstrated that 91% of 118 patients with culture or PCR confirmed EM did not have central clearing.[114]

Further, since a physician witnessed EM was diagnostic for Lyme disease by CDC surveillance case definition at the time this article was published,[109] and there was no

provision in the case definition for a subsequent physician to later refute that diagnosis after the fact without witnessing the rash, then that bell cannot be un-rung. Lastly, Burdge uses a Canadian Lyme serologic standard which is markedly different from the CDC surveillance case definition. Fundamental disparity between these criteria makes it very difficult to draw any balanced conclusions from this piece.

Animal Data

On page 1119, The Guidelines state, *"rare animals may remain culture positive,* [324][115] *and a substantial proportion of animals will remain PCR positive in some,* [325–327][116,117,118] *but not all, studies.*[324][115] *The significance of continued PCR positivity needs to be better understood, but this phenomenon should not necessarily be construed to indicate persistence of viable B. burgdorferi. Unless proven otherwise, culture should be regarded as the gold standard to address viability of B. burgdorferi.*[330, 331][119,120]

The Guidelines reference a single study by Malawista[115] in regard to culture persistence in animals after antibiotic therapy. The "*rare animals*" which remained culture positive were 40% of mice at 60 days post treatment. There were only 5 mice in that treatment group so this study is limited, but to characterize 40% as rare is not accurate.

The Guidelines reference 1 study by ⌘Bockenstedt[116] and 2 studies by Straubinger[117,118] in regard to continued PCR positivity in animals after antibiotic treatment. In addition to the 2 Straubinger's studies cited by The Guidelines,[117,118] a more complete citation of his work documents persistent PCR positivity despite amoxicillin, azithromycin, doxycycline, and ceftriaxone,[117,118,121,122] all four antibiotics having excellent *in vitro* activity against *B. burgdorferi*.

Straubinger's work has also demonstrated that dead borrelial DNA does not persist in uninfected dogs for more than a few days as follows: He observed, "DNA of heat-killed borrelia was not detectable for very long in skin tissue of an uninfected dog, implying that during natural infection the DNA of killed organisms is removed quickly and completely within a few days."[122]

This indicates that a positive PCR result for *B. burgdorferi* equates with active infection. Malawista had separately come to the same conclusion, stating, "*B. burgdorferi* DNA quickly disappeared from tissues" after successful antibiotic therapy.[115] These conclusions are in disagreement with the IDSA statement from above, that *"continued PCR positivity needs to*

be better understood, but this phenomenon should not necessarily be construed to indicate persistence of viable B. burgdorferi."

The study by ⌘Bockenstedt,[116] is inventive and merits further discussion because it is not accurately characterized by The Guidelines. The IDSA references this study only as it pertains to continued PCR positivity in mice after antibiotic therapy for Lyme. Although persuasive, sustained PCR positivity after treatment was not the only important finding in this piece.

This was a xenodiagnosis study, meaning that uninfected ticks were allowed to feed on infected mice that had been treated with either ceftriaxone or doxycycline for 30 days. The ticks were then assessed for the presence of *B. burgdorferi*.

PCR positivity was demonstrated in mice up to 9 months after antibiotic therapy. Moreover, previously uninfected ticks which fed on 10 mice, 5 treated with doxycycline and 5 treated with ceftriaxone, resulted in *B. burgdorferi* infected ticks. Spirochetes were confirmed in ticks that fed from 4 out of 10 (40%) treated mice, 2 out of 5 from both antibiotic treatment groups.

Spirochetes in the ticks were observed under darkfield at both 1 month and 3 months post antibiotic treatment with the same undiminished yield of 40%. All spirochete observations were confirmed as *B. burgdorferi* by outer surface protein A PCR positivity. So in a way this was a culture study as well as a PCR study, but the culturing was done by the ticks.

On page 1119, The Guidelines assert that even though animals remain persistently infected after antibiotics that there is no evidence that clinical findings, i.e. illness, persists as follows: *"The studies also show no evidence for recrudescence or persistence of clinical or histologic findings of an active inflammatory process consistent with B. burgdorferi infection when antibiotic-treated animals are immunosuppressed."*

Regarding the impact of immunosuppression on infected animals, newer data demonstrates recrudescence of *B. burgdorferi* culture positivity after immunosuppression as follows: *B. burgdorferi* has been cultured from anti-tumor necrosis factor-alpha treated mice after previous treatment with ceftriaxone rendered cultures negative.[123]

Yet even without immunosuppression, animal data already referenced by The Guidelines, but not evaluated in its full capacity, demonstrates recurrent illness after antibiotic therapy in dogs. Straubinger found that 1 out of 4 ceftriaxone treated dogs (25%) vs. 2 out of 4

control dogs (50%) continued to have clinically observable episodic lameness after infection.[122] Moreover, it has already been shown that *B. burgdorferi* can cause disease in the absence of histologic findings of active inflammation.[52]

Since none of the animals studies referenced by The Guidelines assessed subjective symptoms after therapy, as has routinely been done in other areas of veterinary medicine,[124,125,126] it is not scientifically rigorous to assume that the animals do not have persistent subjective symptoms caused by their proven chronic infection with *B. burgdorferi*.

Another noteworthy animal study by Hodzic,[127] published after the publication of The Guidelines, builds upon the work of ⌘Bockenstedt. In the Hodzic study, mice were divided into 2 groups by stage of infection; early disease—3 weeks duration; and late disease—4 months duration. All mice were treated with ceftriaxone for 30 days and then examined for persistent infection at 1 and 3 months.

Methods of examination were culture; PCR; histopathology; xenodiagnosis; and allograft transplantation, the latter defined as the transplantation of tissues, from mice that had been infected and then treated, into uninfected mice. Recipient mice were then evaluated for infection by culture & PCR. The results are summarized in Table 1.

Table 1. Hodzic et al.,[127] Determination of persistent infection in antibiotic treated mice

	Pathology(%)	PCR(%)	Xenodiagnosis(%)	Allograft(%)
Early infection-1 month p-tx[a]	1/5(20)	2/5(40)	1/5(20%)	Neg
Early infection-3 month p-tx	Neg[b]	Neg	1/3(33%)	Neg
Late infection-1 month p-tx	3/8(38)[c]	8/8(100)	3/8(38%)	Neg
Late infection-3 month p-tx	1/5(20)	2/5(40)	2/5(40%)	1/5(20%)

[a]p-tx=post-treatment, [b]1/3 Not Done, [c]1/8 Not Done

Of interest is that although none of the treated mice with either early or late disease were culture positive by standard culture methods, 20-40% of ceftriaxone treated mice gave rise to spirochetes within ticks by xenodiagnosis. Of great importance, xenodiagnosis yields increased both with later stage of infection and length of time between treatment and xenodiagnosis. These finding are consistent both with increased rates of chronic persistent infection in treated late stage Lyme disease as compared to early stage disease, as well as continued propagation of infection after discontinuation of antibiotic treatment. Further, 8 out of 9 SCID mice (89%) exposed to xenodiagnosis positive ticks became infected with *B.*

burgdorferi, by either culture or PCR, thus proving continued infectivity of these persisting organisms despite treatment with ceftriaxone.

Hodzic states, "The current study indicated that accessible indices of treatment, such as culture or PCR of skin and serologic response, cannot be relied upon as markers for treatment success. A declining antibody response, as has been noted following antibiotic treatment in mice as well as in antibiotic-treated dogs, occurs despite low levels of persisting spirochetes. Our results show that spirochetes are viable and transmissible and express antigen (based upon immunohistochemistry results) following antibiotic treatment, particularly when commenced during the late stage of infection."[127]

On page 1119, The Guidelines state, *"Possible failure to recapitulate the T>MIC found in humans receiving antibiotic treatment is a potentially serious limitation of almost all of the reported treatment studies of animals."*

No reference is provided for this general statement. In the newer animal studies published after The Guidelines, the authors did specifically address animal pharmacokinetic variables and demonstrated higher antibiotic blood levels than previous animal studies, but still *B. burgdorferi* was cultured after antibiotic therapy.[116,127]

<u>Human PCR Data</u>

On page 1117, The Guidelines reference a study by Bayer, [128] where 74% of 97 American patients with persistent symptoms of Lyme disease despite extensive antibiotic therapy were found to be Lyme PCR positive in urine. The Guidelines state, *"Few additional details were provided by the authors as to the characteristics of the patient population."*

However, the patient population was well characterized in that each had presented with EM following an *Ixodes scapularis* bite in a Lyme endemic area, thus meeting CDC surveillance criteria at the time. Despite multiple rounds of antibiotic therapy before PCR testing, these patients remained chronically symptomatic.

The Guidelines authors concluded that the positive PCR results in the Bayer study "*should be regarded as questionable*", but it is improbable that these results represented false positives since there was not even a single false positive PCR in the negative control group of 62 healthy volunteers. Despite this, in support of its implication that Bayer's results were likely false positives, The Guidelines offer as a comparator a single small study by Rauter of

only 12 patients in which the sensitivity of *B. burgdorferi* urine PCR for Lyme patients with EM was 8%.[129]

Despite the fact that the Bayer and Rauter studies focus on Lyme urine PCR, they are otherwise apples and oranges. First, the much larger Bayer study used twice as many PCR primers as the smaller Rauter study. Second, the Bayer study participants had chronic Lyme disease whereas the Rauter study participants had EM. Disseminated Lyme in animal models results in 93% of mice developing Lyme cystitis;[130] and whereas histologically proven Lyme cystitis in humans has been documented in late Lyme disease,[157] it is unlikely that patients with EM stage disease would yet have the opportunity to develop Lyme cystitis.

Consistent with these findings, before The Guidelines were published it had already been established that "…PCR as performed by two different primers and probes is not sensitive enough to detect the few borreliae present in urine from patients with erythema migrans.[131]" As such, it is inappropriate that The Guidelines authors had chosen the Rauter study as evidence, especially since a more representative comparison to the Bayer study had already been published by Pícha.[132]

In the larger Pícha study of 57 well characterized patients with active neuroborreliosis and intrathecal antibody production, Lyme urine PCR using 3 targets resulted in a sensitivity of 49%, which was higher than their CSF PCR sensitivity of 35%.[132] It is very unlikely that urine PCR positivity in this study was due to false positives since serial urine PCR's significantly declined with antibiotic treatment. More recent studies continue to agree with and support Bayer's original findings in that by using even more primers, PCR sensitivity can be increased. For example, researchers demonstrate that using 5 primers in conjunction with testing several body fluids results in consistently increased PCR sensitivities.[133]

On page 1107, The Guidelines state, *"Amplification of B. burgdorferi DNA in CSF using PCR by a laboratory with excellent quality control can also be useful* [103, 124, 167], [168,134,135] *but few laboratories are capable of accurately performing this test."*

The Guidelines authors offer no reference for their admonition that *"few laboratories are capable of accurately performing this test."* The widely accepted reality is that PCR as a technology has been around for several decades. Innumerable CLIA certified laboratories are capable of performing such tests.[136] The problem is not in the performance of this technology. As such, false positivity for PCR due to contamination is quite low in general, resulting in uniformly high specificity.[168] Rather, the problem lies with the complex microbiology of *B. burgdorferi*.

On page 1112, The Guidelines authors state, *"…PCR testing of serial joint fluid samples suggest that arthritis may persist in a small number of patients, despite apparent eradication of the spirochete (i.e., absence of amplifiable B. burgdorferi DNA by PCR)."* The Guidelines define such persistent arthritis as "*antibiotic-refractory Lyme arthritis."* On page 1113, they recommend symptomatic therapy for this condition.

This statement portrays a negative *B. burgdorferi* PCR from a treated patient as good evidence of lack of active infection. However, it is well documented that in patients with "*antibiotic-refractory Lyme arthritis*," even if synovial fluid had been Lyme PCR positive and subsequently becomes Lyme PCR negative after antibiotic therapy, this does not necessarily equate with absence of infection.[165] Such patients, who can remain synovial membrane Lyme PCR positive but synovial fluid PCR negative, respond to more aggressive antibiotic re-treatment.[165]

From a variety of body fluids, Lyme PCR has well recognized problems with lack of *in vivo* sensitivity, especially when using only one or two primers with too few samples and/or sample types. As such, ⌘Stanek states, "Negative PCR does not rule out diagnosis of Lyme disease…Direct detection of the pathogen is unsuitable for the monitoring of treatment success because a negative result does not rule out persistence of the pathogen.[137]" In agreement, ⌘Dumler states, "DNA from plasma samples was highly insensitive…[138]"

In light of the foregoing, it seems that the IDSA apportions undue emphasis as to the predictive value of a negative Lyme PCR. As such, a negative result does not adequately rule out infection. A positive result however does indicate active infection. This is acknowledged by The Guidelines authors as follows:

> **Page 1121: "*Table 5. Proposed definition of post–Lyme disease syndrome*"**
> *"Although testing by either culture or PCR for evidence of Borrelia burgdorferi infection is not required, should such testing be done by reliable methods, a positive result would be an exclusion."*

On page 1110, The Guidelines state, *"Positive PCR results for a joint fluid specimen from a seronegative patient, however, should be regarded with skepticism."*

This is an unanticipated warning since ⌘Wormser reported that the specificity of synovial fluid Lyme PCR is 100%, as averaged from over 8 different studies from both the United States and Europe.[168] Moreover, seronegative Lyme arthritis with a positive synovial fluid

PCR has been reported in 45% of patients in some studies.[133] Further, in Table 5 of The Guidelines quoted directly above, a positive Lyme PCR is thought to have strong diagnostic value, so their inconsistent view on this matter is puzzling.

While evaluating neurologic Lyme disease patients by PCR, ⌘Strle demonstrated Lyme PCR positivity in his control group of patients with tick-borne encephalitis (TBE), but emphatically denied that they were false positives as follows: "…We emphasize that all contamination precautions were strictly followed, that all routine negative controls gave negative results, and that the quality of the samples was monitored.[139]" Clearly, patients with TBE may not have been the wisest choice for a control group since they are also at risk for Lyme disease given the fact that *B. burgdorferi* infection in Ixodes ricinus in Slovenia is 25-100 times more common that TBE virus.[139] In the end, ⌘Strle found Lyme PCR indicative of *B. burgdorferi* infection in his control group, when other diagnostic tools were unrevealing.

In light of the foregoing, The Guidelines authors tend to view positive Lyme PCR results with inappropriate skepticism. This is incongruous since they characterize a positive Lyme PCR as *"evidence of Borrelia burgdorferi infection"* elsewhere in The Guidelines (Table 5); and in their previously published work, which has also demonstrated excellent specificity.[168]

Human Culture Data

On page 1117, The Guidelines reference two small studies of 13 patients each, citing previously culture positive erythema migrans which were then culture negative after treatment,[140,141] implying that a negative culture result after treatment is a material finding.

Refuting this implication, ⌘Wormser writes, "… culture is useful only for untreated patients. Culture positivity is rapidly aborted by even a few doses of appropriate antibiotic treatment."[168] As such, a negative culture after even a sub-therapeutic antibiotic treatment is the expected outcome. It does not necessarily correlate with cure and cannot be taken as material proof of absence of infection. On the other hand, a positive *B. burgdorferi* culture after treatment would be a material finding.

On page 1118, The Guidelines reference a study by Hunfeld and ⌘Strle[142] where 19 of 1148 patients (1.7%) with antibiotic treated EM demonstrated growth of *B. burgdorferi* from normal appearing skin in the area of the prior EM's. In 5 of the 19 samples where organisms were available for direct comparison, 1 was identical to the initial isolate, 3 were of the same species but demonstrated different plasmid and other typing methods, and 1 was of a

different species. The IDSA suggests that these findings are likely due to re-infection or contamination.

Regarding the culture which proved identical to its original paired isolate, given the massive strain heterogeneity among wild type *B. burgdorferi*, the odds of re-infection with an identical strain are miniscule, and especially at the identical spot in the skin as the first infection. Contamination with the very same strain would be likewise improbable. Further, ⌘Strle previously penned two similar studies culturing *B. burgdorferi* from normal appearing skin in the area of prior treated, culture confirmed, EM. The average failure rate of antibiotic therapy in those studies ranged from 2%-18%,[143,144] roughly equal to, or greater than, Hunfeld's and ⌘Strle's finding of 1.7%; as such, it further decreases the likelihood of contamination in the Hunfeld and ⌘Strle study. [142]

Even with different strains and species, the possibility of re-infection at the exact same spot of skin is remote. It is logically invalid to suggest that within a few weeks time there would be any reasonable probability that a tick would just happen to bite in the exact same location as the prior EM. As evidence, ⌘Krause found that in re-infection, virtually all 2nd EM's were at different parts of the body than the first EM.[145]

A more likely scenario to explain plasmid differences for the 3 different strains of the same species is genetic recombination, which has been well established in *B. burgdorferi*.[146] Another scenario that can explain the findings of both strain and species differences is heterogeneity of the initial infections. Before The Guidelines were published, various studies had already shown that Lyme disease patients frequently become infected concurrently with multiple strains and species of *B. burgdorferi.*

For example, ⌘Strle had already demonstrated that in 50 patients from whom 2 different *B. burgdorferi* cultures were isolated from distinct areas of the body, 24% of patients were simultaneously infected with different strains, whereas 6% were concomitantly infected with entirely different species.[147] Along these same lines, ⌘Dattwyler had demonstrated simultaneous multi-strain infection with *B. burgdorferi* sensu stricto in EM patients.[148] Although both of these studies were published before the publication of The Guidelines, neither was referenced by The Guidelines.

On page 1118, The Guidelines state, "*Culture contamination would be consistent with the absence of clinical findings at the skin site, the observation that the rate of positive culture results after repeated biopsy was similar regardless of which antibiotic class the patient had received for treatment (F.S. [⌘Franc Strle] unpublished data) [313]*[3] *and the lack of antibiotic*

resistance in the reisolated borrelial strains[310,313][3,142] *Culture contamination has occurred before in laboratories growing B. burgdorferi (G.W. [⌘Gary Wormser], unpublished data).*"

To address these claims in order, first, EM typically resolves spontaneously even without antibiotic therapy, yet *B. burgdorferi* infection persists, so their first point is moot. Second, the rate of positive culture being invariable in regard to antibiotic class has no bearing as follows: In the Hunfeld and ⌘Strle article,[142] it states, "As shown in this study and demonstrated earlier by Preac-Mursic et al., isolates can also differ in their individual susceptibilities to various antimicrobial agents. These minor differences, however, are of no clinical relevance, as they commonly do not exceed the critical concentrations for these substances to become ineffective and therefore cannot explain survival of spirochetes during prolonged effective antibiotic therapy." Third, the IDSA claims lack of antibiotic resistance of the post-treatment isolates as proof of contamination, yet the article they reference by ⌘Strle shows very little in terms of antibiotic resistance in post antibiotic therapy isolates.[3] Then in the very next paragraph they claim that antibiotic resistance in the entire genus does not even exist, thus invalidating their claim.

However, although not demonstrated in the Hunfeld and ⌘Strle study,[142] antibiotic resistance has sometimes been documented in *B. burgdorferi*. Hunfeld and ⌘Strle conclude that antibiotic resistance was not the mechanism for persistence in their study, but that other mechanisms were responsible. More recent data demonstrates that the extracellular matrix also provides sanctuary for *B. burgdorferi*.[149]

Lastly, The Guidelines authors claim that culture contamination can occur per unpublished anecdotal data by ⌘Wormser. As with any culture for any infectious disease, there is no reason to believe that it could not occur. To prevent this, Hunfeld and ⌘Strle document their good laboratory hygiene, stating, "All subcultures were monitored for vitality of spirochetes and possible contamination by conventional dark-field microscopy."[142]

At the time of publication of The Guidelines, there was not even a single published instance documenting even a single case of *B. burgdorferi* culture contamination, ever. So for the IDSA to uniformly assume culture contamination for all instances of culture proven persistent infection after treatment is not scientifically rigorous. Since publication of The Guidelines, there has been one occurrence of *B. burgdorferi* culture contamination, and this continues to be the only documented case in the whole of medical literature.[189] So given the extreme rarity of a false positive, it is most appropriate to assume that a positive *B. burgdorferi* culture is a true positive.

On page 1118, The Guidelines reference two studies where *B. burgdorferi* was cultured from patients with Lyme disease despite antibiotic therapy[54,150] and refers to them as "anecdotal instances".

In the first study by Preac-Mursic,[54] the clinical histories were explored of 6 patients with predominantly ocular manifestations of late Lyme disease who all had positive cultures for *B. burgdorferi*. Cultures were confirmed by both sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and binding to specific monoclonal antibodies which react against *B. burgdorferi*. Clinical histories are depicted in Table 2.

Table 2. Preac-Mursic et al.,[54] Positive *B. burgdorferi* culture despite antibiotics in Ocular Lyme

Pt[a]	Disease		Antibiotics Taken Active Against Bb[f]	Lyme Antibodies				Positive Culture Source
				Serum		CSF		
	Systemic	Ocular		IgM	IgG	IgM	IgG	
1	None	Panuveitis-Iridocyclitis	Doxy 8 wks total[c] 2 courses of treatment	NEG	POS	ND	ND	Iris
2	EM in the past	Iritis-Uveitis	Doxy 10 d Ceftriaxone 3 wks[d]	NEG	NEG	ND	ND	Skin
3	Tinnitus	None	Cefotaxime 5 d[d]	NEG	POS	NEG	NEG	CSF
4	None	Painful eyes	Ceftriaxone 14 d[d]	NEG	POS	NEG	NEG	CSF
5	Arthralgias Lymphad.[b]	Conjunctivitis	PO Penicillin 24 d total 2 courses of treatment Ceftriaxone 14 d[e]	NEG	NEG	NEG	NEG	CSF
6	Radicular Pain	Iritis	Ceftriaxone 14d[d]	NEG	POS	NEG	NEG	CSF

[a]Pt=patient, [b]Lymphad.=lymphadenopathy, [c]Both courses of doxycycline given before positive biopsy, [d]Antibiotics given after biopsy, [e]Both courses of oral penicillin given before biopsy, ceftriaxone given after biopsy, [f]Bb=B. burgdorferi,

This study demonstrates persistent infection with *B. burgdorferi* despite prior antibiotic therapy. Patient #1 had received doxycycline for 4 weeks, twice, so a total of 8 weeks, and patient #5 had received oral penicillin for 12 days, twice, so a total of 24 days. It also documents *B. burgdorferi* in the CSF of seronegative as well as CSF Lyme antibody negative patients. It further documents persistent infection in late Lyme disease patients who have subjective symptoms and/or objective signs not described by CDC surveillance case definition.

In the second study by Preac-Mursic,[150] the experiences of 6 patients were chronicled. *B. burgdorferi* culture was positive from all patients after antibiotic therapy. Spirochetal isolates were confirmed as *B. burgdorferi* by SDS-PAGE analysis. Clinical histories are illustrated in Table 3.

Table 3. Preac-Mursic et al.,[150] Positive *B. burgdorferi* culture despite antibiotics

Patient #	Antibiotics Before Culture	Cx[d] Source, Months After Treatment	Lyme Antibody Status--Time of Culture		
			IgM Blood	IgG Blood	CSF
1 Rad[a]	PO Pen[b] 14d, Doxy 10 d	CSF, 7 months	POS	POS	NEG
2 Rad	IV Pen 10 d	CSF, 3 months	POS	POS	NEG
3 Rad	IV ceftriaxone 10d	CSF, 7.5 months.	Not Done	Not Done	Not Done
4 EM	PO Pen 12d	Skin biopsy, 3 months	POS	POS	Not Done
5 EM	IV Pen 10 d	Skin biopsy, 2 months	POS	NEG	Not Done
6 EM	Doxy[c] 10 d	Skin biopsy, 1 month	NEG	NEG	Not Done

[a]Rad=Radiculitis, [b]Pen=penicillin, [c]Doxy=doxycycline, [d]Cx=Culture

Patient #1 initially had CSF pleocytosis before treatment, but was negative for CSF Lyme antibody, and culture was not initially performed. After doxycycline, CSF pleocytosis resolved. At the time of positive CSF culture and clinical relapse 7 months later, CSF again showed pleocytosis, but Lyme antibodies continued to be negative in CSF throughout.

Patient #2 had initial CSF pleocytosis before treatment, but neither Lyme antibody nor culture was performed. He then had a second lumbar puncture 4 days after antibiotic therapy and still had pleocytosis, as well as positive Lyme antibodies in CSF, but a culture was not performed. Three months later, at the time of positive CSF culture, CSF was negative for both pleocytosis and Lyme antibodies. The patient was asymptomatic.

This study illustrates several important findings. First, it documents persistent infection with *B. burgdorferi* despite antibiotic therapy. Second, it documents *B. burgdorferi* cultured from normal appearing CSF without Lyme antibodies. Third, it documents asymptomatic chronic *B. burgdorferi* infection.

Given that ⌘Wormser had previously written, "…culture of *B. burgdorferi* **sensu lato** undoubtedly offers the best confirmation of active infection…,[168]" it comes as a surprise that The Guidelines would not express more interest in these two positive culture studies.[54,150]

On page 1118, instead, The Guidelines respond to this research[54,150] by stating, "In none of the studies, however, could reinfection or laboratory contamination be excluded."

The odds of re-infection would be slim. In the first study, the authors specifically address this by stating, "The lack of repeated insect bite and erythema migrans, negative AB-titers against *B. burgdorferi* and negative CSF examination suggest persistence of *B. burgdorferi*

rather than reinfection.[54]" In the second study, in most cases cultures were performed 1-3 months after antibiotic therapy.[150] The chance of re-infection occurring in that small time frame would be small.

In regard to contamination, the prospect exists for any culture but certainly less so for *B. burgdorferi*, given how difficult it is to cultivate in the first place.[168] It is inexplicable that The Guidelines authors would assume contamination for these studies,[54,150] when at the time of The Guidelines publication, there had never been a single published case of *B. burgdorferi* culture contamination. As evidence of just how fastidious this organism can be, it took up to 16 subcultures to achieve culture success.[54]

B. Further Evidence Against the Contested Recommendation—Either Not Referenced by The Guidelines, or Not Referenced in Full Capacity

Human PCR and/or Histopathology

In a study coauthored by ⌘Steere, PCR results were disclosed of Lyme arthritis patients treated with multiple courses of antibiotics.[151] Of 43 patients treated for Lyme arthritis, 2 groups were described as follows in Table 4.

Table 4. Nocton et al.,[151] Chronic Lyme arthritis despite antibiotics

Patients	Treatment Received	SF[c] PCR Positive After Treatment
19	30-60d PO antibiotics and/or IV up to 3 wks	7 out of 19 (37%)
10[a]	"multiple courses of antibiotic therapy"[b]	3 out of 10 (30%)

[a] 10 of 43 treated patients (23%) developed "chronic Lyme arthritis" despite "multiple courses" of antibiotics, [b] the authors did not disclose additional details regarding antibiotic therapy, [c]SF=synovial fluid

This study by ⌘Steere documents persistent infection with *B. burgdorferi* despite multiple courses of antibiotic therapy recommended for Lyme disease. Since patients in this study were evaluated in Connecticut and local disease acquisition is probable, these findings most likely represent *B. burgdorferi* **sensu stricto**. This study was referenced by The Guidelines, but not in its full capacity, in that demonstration of persistent *B. burgdorferi* PCR positivity after antibiotics was not mentioned.

In another study coauthored by ⌘Steere, a retrospective cohort analysis was performed of 38 American Lyme disease patients, and 43 controls, to determine the long term outcomes of treated Lyme disease.[152]

Ten of 38 patients (26%) relapsed within one year after treatment and required re-treatment with antibiotics. The relapses which prompted antibiotic re-treatment consisted of "fatigue, persistent arthritis or arthralgias, headaches, or difficulty with memory and concentration" and were not limited to objective features only.

At a mean of 6.2 years after initial Lyme disease, despite the first course of antibiotic therapy and all antibiotic re-treatments, in sum the Lyme group still had increased symptoms consisting of arthralgias, distal paresthesias, concentration difficulties, fatigue, abnormal joints, verbal memory deficits, and poorer global health scores as compared to controls. More precisely, 13 of 38 patients (34%) had long term pathology consisting of arthritis or recurrent arthralgias, neurocognitive impairment, neuropathy, or myelopathy.

Patient #12 had been initially treated with 2 weeks of IV penicillin. Despite therapy, she developed severe neurologic illness diagnosed as supranuclear palsy. Her CSF was negative for Lyme antibodies. She was re-treated with 2 weeks of IV ceftriaxone without effect. The patient died. Autopsy revealed 2 spirochetes in her brain by silver stain as well as some mononuclear inflammation.

In this American patient with well-documented Lyme disease, most likely locally acquired, this study demonstrates persistent infection with *B. burgdorferi*, most likely **sensu stricto,** with central nervous system invasion despite negative Lyme antibodies in CSF.

It is possible that *B. burgdorferi* infection may have caused this patient's fatal illness, as there is at least one other published case of *B. burgdorferi* infection causing progressive supranuclear palsy, which unlike this case, was responsive to ceftriaxone.[153] Although this study was referenced by The Guidelines, no mention was made of the observed persistence of spirochetes in brain tissue after failure of IV penicillin and IV ceftriaxone.

In another study by ⌘Steere, the synovial membranes of 12 patients with Lyme arthritis refractory to antibiotic therapy were extracted during therapeutic synovectomies.[154] Despite antibiotic therapy, intact spirochetes were demonstrated in the synovial tissue of 6 out of 12 patients (50%). The details of antibiotic treatment were not disclosed in the study. ⌘Steere wrote, "…the antigenic stimulus in Lyme arthritis would appear to be a small number of live spirochetes, demonstrated here by monoclonal antibodies, which may persist in the synovial

lesion for years." This study demonstrates the persistence of *B. burgdorferi* despite antibiotic therapy, most likely **sensu stricto** in this American study. This study was not referenced by The Guidelines.

In another study coauthored by ⌘Steere, the clinical history was detailed of a 67 year-old woman who died from adult respiratory distress syndrome due to seropositive Lyme disease acquired in the USA.[155] She failed a 2 week course of tetracycline, a 10 day course of IV penicillin, and another course of IV penicillin, duration unspecified. Of note, there were partial improvements associated with the 2 courses of IV penicillin and worsening associated with the administration of corticosteroids.

On autopsy, lymph nodes revealed spirochetes by silver stain that were consistent with *B. burgdorferi*. In a patient with disease acquisition in the USA, this study documents persistent and ultimately fatal infection with *B. burgdorferi* **sensu stricto** despite multiple courses of IV and oral antibiotic therapy for Lyme disease. It highlights the possible risks of corticosteroid use in Lyme disease patients. This study was not referenced by The Guidelines.

In a study by Battafrano, a 24 year old woman presented with EM after camping in Pennsylvania.[156] Several years later she developed migratory arthritis which persisted for years, requiring multiple surgical procedures. Ultimately, Lyme arthritis was diagnosed with positive Lyme serologies. Two courses of IV penicillin, 3 courses of IV ceftriaxone, and one course of IM penicillin all resulted in "dramatic reduction" of arthritis, but after discontinuation of each course, arthritis recurred. Doxycycline for 13 continuous months did not provide benefit. Sulfasalazine for one year likewise provided no benefit. Therapeutic arthroscopic synovectomy was performed without benefit. Silver stain of synovial tissue and synovial fluid revealed numerous spirochetes and *B. burgdorferi* PCR of synovial fluid was positive.

This study documents chronic persistent infection with *B. burgdorferi* **sensu stricto** (Lyme acquisition documented in Pennsylvania) over many years despite long term, repeated courses of aggressive IV antibiotic therapy. Second, it demonstrates multiple temporary but "dramatic" responses to beta-lactam antibiotics which do not possess clear anti-inflammatory properties. Yet there was no response to doxycycline, which does possess some anti-inflammatory properties. Further along these lines, the patient likewise had no response to sulfasalazine, a powerful immune suppressant. In sum, this emphasizes that transient responses to antibiotic therapy in chronic Lyme disease are most likely due to a partially

treated refractory infection rather than an anti-inflammatory effect from antibiotics. This study was not referenced by The Guidelines.

In a study by Liegner, the clinical history is revealed of a 68 year-old woman from New York City who developed EM associated with myalgias and arthralgias 3 weeks after a hiking excursion in Rockland County, NY.[46] Lyme serologies were negative. She was treated for Lyme disease with tetracycline for 10 days which resulted in resolution of joint symptoms and partial fading of the rash. Four months later, the rash was still there and physical exam revealed joint tenderness. The patient was re-treated with minocycline for 3 months. The rash faded further with this treatment but still slightly remained. Fleeting arthralgias continued after treatment but eventually subsided.

Two months later, she began to experience migratory quick stabbing pains which resolved after about one month. Lyme serologies were again negative. Three months after that, with no opportunity for re-exposure to *Ixodes scapularis* while living in New York City, the original EM increased in intensity and a new annular rash developed suggestive of EM, all in association with the recurrence of joint pain. At that time, a whole blood Lyme PCR was positive, which was then repeated again with the same positive results. A biopsy of the rash demonstrated histologic findings compatible with EM and a spirochetal structure was demonstrated with silver stain. Culture was negative.

Minocycline treatment was resumed and the lesions faded. At 3 months treatment, PCR testing was negative. Lyme serologies were still negative by 2-tiered CDC surveillance case definition, but a Western blot demonstrated weak banding at IgG 41,31,66 and IgM 31. The patient continued minocycline treatment for 10 months and was completely well. She remained well at long term follow up 9 months later.

This article depicts several important findings. First, it documents persistence of *B. burgdorferi* **sensu stricto** despite antibiotic therapy for 3 months in a seronegative EM patient whose Lyme disease was acquired in the United States. Second, it demonstrates that an incomplete clinical response to treatment was associated with persistent infection and subsequent relapse. Third, it documents that although EM and associated clinical features of disseminated Lyme persisted despite 3 months of antibiotic therapy, full resolution was achieved with longer term, i.e. 10 months, antibiotic therapy. This study was not referenced by The Guidelines.

In a study by Chancellor, the clinical histories of 7 patients with neurologic and urologic presentations of Lyme disease are discussed.[157] All patients were initially treated with IV ceftriaxone for a mean of 3 weeks, range 2-5 weeks, but 4 of 7 patients (57%) relapsed despite this treatment. Relapsed patients were re-treated with IV ceftriaxone for a mean of 2.75 weeks, range 2-3 weeks. Even though antibiotic treatment was helpful in all cases, 5 of 7 patients (71%) remained symptomatic. Of the 2 patients who eventually became asymptomatic, 1 had previously relapsed and required re-treatment.

In patient #2, who relapsed despite initial treatment with IV ceftriaxone for 3 weeks, spirochetes compatible with *B. burgdorferi* were demonstrated by silver stain in a bladder biopsy. These were confirmed as *B. burgdorferi* with monoclonal antibodies.

This article documents the persistence of *B. burgdorferi*, most likely **sensu stricto** in this American study, despite IV ceftriaxone for 3 weeks. Second, it documents that clinical relapses are common after antibiotic therapy, and that they respond, at least partially and sometimes fully, to antibiotic re-treatment. Third, it demonstrates that even though antibiotic treatment was helpful in all cases, 71% of patients did not achieve full recovery. <u>This study was not referenced by The Guidelines.</u>

In a study by Svecová,[158] the clinical history was presented of a 73-year old woman who had five episodes of EM, with Lyme serologies evolving to positive, despite repeated courses of antibiotic therapy. The patient had visited a Lyme endemic area only once before manifesting the first EM. There was no history of tick bite and at any time and there was no history of potential re-exposure after the first episode of EM.

The 1st episode of EM was on the buttock and the patient was treated with doxycycline for ten days with resolution of the rash. Then, 10 months later, the second episode of EM recurred in the same spot as the first EM. She was treated with azithromycin for 5 days with resolution of the rash. Then, 9 months later, a 3rd EM manifested on the left forearm. She was treated with amoxicillin for 21 days with resolution of the rash. Then 10 months later, a 4th episode of EM occurred on the right forearm. At this time, a Lyme PCR of the blood was checked and found to be positive. The patient was treated with IV ceftriaxone for 21 days. During the 2nd day of ceftriaxone, a 5th episode of EM occurred in the same spot as both the 1st and 2nd episodes of EM. Recurrence of an original EM has been described elsewhere in chronic Lyme disease,[46] as have rashes during the second day of antibiotic therapy consistent with a cutaneous herxheimer.[177] Further, recurrent herxheimer reactions have been well described upon re-initiation of repeated courses of antibiotics in chronic Lyme disease,[25] and does not implicate re-infection.

This study most likely represents persistent infection rather than re-infection as the cause of the recurrent episodes of EM for the following reasons: First, recurrent EM's without an opportunity for re-exposure are suggestive of persistent infection rather than re-infection. Second, the 1st, 2nd, and 5th episodes of EM were all in the same spot, which would be unlikely in the event of re-infection. Third, the 3rd and 4th episodes of EM were on the forearms. Tick bites in highly visible areas such as these would be fairly conspicuous, but none were recalled. Fourth, unlike American patients with EM who recall a tick bite only 25% of the time, ⌘Wormser, ⌘Nadelman, and ⌘Strle have jointly published that European patients with EM recall a tick bite 64% of the time.[159] Therefore, the odds that this patient had been re-infected 4 additional times after the initial EM and had not recalled *any* of the 4 tick bites would be 1.7%. The math is calculated as follows: Odds of a European patient *not* recalling a tick bite associated with EM is {1–.64} = .36 or 36%.[159] The odds of this happening 4 times in a row would be $.36^4$ = .017 or 1.7%

This study documents the persistence of *B. burgdorferi* in an EM patient despite repeated courses of antibiotic therapy. Per the above discussion, the greater likelihood is that this patient had remained persistently infected despite these multiple rounds of antibiotic therapy. <u>This study was published *after* publication of The Guidelines.</u>

In a study by Cimmino,[160] a 54 year old man presented to the hospital with a 2 year history of intermittent chronic fevers, arthralgias, sore throat, and multiple transient non-pruritic circular rashes, each measuring 2-4cm, biopsy of which demonstrated "non-specific lymphocytic vasculitis." During his hospitalization splenomegaly was also found. Ultimately, after a long work up the patient was treated with prednisone for the "presumptive diagnosis of adult-onset Stills disease."

The patient worsened during the corticosteroid taper and was re-admitted to the hospital. Lyme serologies were positive at that time. The patient was treated with IV penicillin and experienced a transient worsening of symptoms in the first few days. After 3 weeks of this therapy, the patient was still febrile and anemic. Splenectomy was performed. Spirochetes compatible with *B. burgdorferi* were demonstrated in the spleen. The patient was re-treated with IV penicillin with modest benefit. At attempt was made to treat him with ceftriaxone, but he had an allergic reaction after a single dose.

The study documents persistent infection with *B. burgdorferi* despite IV penicillin. It illustrates the association of corticosteroid use before antibiotic therapy with ultimate antibiotic treatment failure. <u>This study was not referenced by The Guidelines.</u>

In a study by Frey, 8 consecutive patients were examined who originally met CDC surveillance case definition for Lyme disease. The patients then developed widespread chronic myalgia which began either immediately with or after Lyme disease.[161] Seven out of 8 patients (88%) were treated with antibiotics. Six out of 8 (75%) fulfilled American College of Rheumatology diagnostic criteria for fibromyalgia. The remaining 2 of 8 patients (25%) had generalized myalgia but less than 11 trigger points. Muscle biopsies were performed. At the time of biopsy, 5 of 7 antibiotic treated but persistently symptomatic patients (71%) were seronegative; 3 of 7 antibiotic treated but persistently symptomatic patients (43%) were Lyme PCR positive in the muscle; and 1 seropositive untreated symptomatic patient (#7) was PCR positive as well. This study was performed under strict quality control in 3 different laboratory rooms where *B. burgdorferi* had never been cultured before. All 14 human negative controls whose muscle biopsies were obtained during orthopedic procedures yielded negative Lyme PCR results. See Tables 5 & 6.

Table 5. Frey et al.,[161] Persistent myalgia despite antibiotics—Clinical history

Patients	Time From Lyme Onset to Myalgia	Myalgia Duration Before Muscle Biopsy	Antibiotics Before Muscle Biopsy	Time From Antibiotic to Muscle Biopsy
#1	12 months	18 months	Doxycycline 14d, Ceftriaxone 14d	16 months
#2	3 months	3 months	Ceftriaxone 21d	3 months
#3	immediate	40 months	Ceftriaxone 14d	38 months
#4	12 months	60 months	Ceftriaxone 21d, Minocycline 90d, Amoxicillin 14d	19 months
#5	1 months	5 months	Amoxicillin 30d	5 months
#6	4 months	33 months	Amoxicillin 14d, Ceftriaxone 21d Doxycycline 21d	3 months
#7	immediate	24 months	No treatment	N/A
#8	immediate	6 months	Amoxicillin 15d, Minocycline 12d	3 months

Table 6. Frey et al.,[161] Myalgia despite antibiotics—Seronegative, Positive Lyme PCR

Patients	IgG Titers	IgG Western Blot	Lyme PCR Muscle
#1	Negative	Negative	Negative
#2	**Positive**	**Positive**	Negative
#3	Negative	**Positive**	**Positive**
#4	**Positive**	**Positive**	Negative
#5	Negative	Negative	Negative
#6	Negative	Negative	**Positive**
#7	**Positive**	**Positive**	**Positive**
#8	Negative	Negative	**Positive**

This observational study demonstrates several important findings. First, it documents the persistence of *B. burgdorferi* in chronic Lyme disease patients who manifest only subjective symptoms not described by CDC surveillance case definition. Second, persistent infection is documented despite recommended oral and intravenous antibiotic treatment regimens. Third, chronic myalgia began after Lyme disease, with most patients meeting American College of Rheumatology diagnostic criteria for fibromyalgia. Fourth, it demonstrates persistent infection in seronegative chronic Lyme disease.

Since *B. burgdorferi* DNA was isolated from the muscles of these patients, it is most likely that persistent infection was responsible for their myalgia. This study supports the finding that "post-Lyme fibromyalgia" is a misnomer, that this condition is likely caused by persistent infection with *B. burgdorferi*. This study was not referenced by The Guidelines.

In a study by Lawrence, the clinical history of a previously healthy 58 year-old woman was published.[25] This seronegative patient, without history of tick bite or EM, developed multiple neurologic signs and symptoms. Table 7 illustrates her lumbar puncture results according to her treatment timeline.

Table 7. Lawrence et al.,[25] Chronic seronegative Lyme disease—Lumbar puncture analyses

LP Timeline	Protein	WBC	PCR	CSF Ab[b]	Osp A[c]	Bb IC[d]
Initial	56	3			POS	
Before 1st Rx[a]	50	0				
Before 2nd Rx	40	0	POS		POS	
Before 3rd Rx	38	0	POS	IgA POS IgG NEG	POS	POS
36 hrs after 3rd Rx	42	0	POS		POS	POS
Before 4th Rx	75	0			POS	
Before 6th Rx	56	1	POS		POS	POS

[a]Rx=antibiotic treatment, [b]CSF Ab=cerebrospinal fluid Lyme antibodies, always negative for free IgG per the text of the article; only a single finding of intrathecal Lyme IgA antibody , [c]Osp A= Outer surface protein A antigen detected in cerebrospinal fluid, [d]Bb IC=B. burgdorferi specific immune complexes detected in cerebrospinal fluid

The patient was treated for seronegative Lyme disease with IV ceftriaxone for 3 weeks. Within 12 hours of the first dose the authors observed, "the patient became confused, and then stuporous, with a temperature of 39.2 °C. The time course was consistent with a J-H [Jarisch-Herxheimer] and those symptoms resolved spontaneously in 48h." She improved with ceftriaxone.

Three months later she developed uveitis, keratitis, pulsatile tinnitus, atrophy of the tongue, and loss of taste. She was re-treated with IV ceftriaxone for 8 weeks. The authors state, "Another J-H reaction occurred 24 h after starting antibiotic and then spontaneously cleared." The patient improved and returned to work.

Six months later, she experienced right retro-orbital pain, right temporal artery tenderness, and positive visual evoked potentials. Two months after that, she developed progressive right hemiparesis. She was re-treated a third time with IV ceftriaxone. The authors wrote, "twenty-one hours after antibiotic was started she developed blurred vision in her right eye followed by stupor. This was again considered to be a J-H reaction…Visual acuity returned to normal within 4 days." The patient was treated this time with ceftriaxone for 2 weeks followed up directly with doxycycline for 19 weeks. She improved and remained well while on doxycycline.

Within 2 weeks of stopping doxycycline, the patient experienced vertigo. Two months later, she experienced paresthesias of the face and numbness inside her mouth. The patient was treated with a fourth course of IV antibiotics, this time cefotaxime. The authors state, "Within 24 h of starting antibiotics, she developed multifocal myoclonic jerks and became unresponsive, with a dense right hemiparesis. This J-H reaction occurred despite pre-medication with 80 mg prednisone followed by 20mg every 6h." Twelve hours later the patient improved. Three weeks into cefotaxime, neutropenia occurred, so cefotaxime was discontinued and changed to doxycycline but the patient had increasing neurologic symptoms on this course. The authors did not specify how long the doxycycline was given.

Approximately 6 months later, the patient was re-treated. The authors did not specify what neurologic symptoms the patient was experiencing at that time, but it had been specified that she did not recover well after the previous doxycycline. The patient was treated for the 5th time with IV antibiotics, this time ceftriaxone. She was pre-medicated with corticosteroids, but still experienced a transient flare of symptoms upon re-starting antibiotics, characterized by the authors as a Jarisch-Herxheimer reaction. She continued with ceftriaxone for 2 weeks followed by 9 "pulsed" doses of IV ceftriaxone 3gm/day 2 days per week. This was discontinued due to possible serum sickness reactions. The authors did not specify whether or not this therapy was beneficial.

Approximately 6 months after that, the patient had findings compatible with mononeuritis multiplex. The patient was re-treated with a 6th course of IV antibiotics, ceftriaxone again. This time she was treated with ceftriaxone for 2 weeks followed up with long term clarithromycin. She accrued benefits from antibiotic treatment which persisted while being

maintained on antibiotic therapy. At the time the article was written, the patient had been on clarithromycin for 22 months without recurrence of symptoms during that time.

This paper demonstrates numerous significant findings. First, it illustrates both on clinical and laboratory grounds, persistent infection with *B. burgdorferi* despite repeated long term courses of very aggressive oral and IV antibiotic therapy. Second, it documents persistent infection in seronegative chronic Lyme disease. Third, it documents persistent infection and recurrent neurologic symptoms in the absence of Lyme IgG antibodies in CSF. Fourth, it documents the predominance of subjective symptoms and objective signs not described by CDC surveillance case definition in this patient with persistent infection. Fifth, it documents the existence and clinical relevance of repeated Herxheimer reactions in chronic Lyme disease; as this reaction relates to the treatment of active *B. burgdorferi* infection. Sixth, it demonstrates that even very aggressive, long term antibiotic treatment does not necessarily result in complete cure; however, treatment did help very significantly and during the last long term treatment with 22 months of continuous clarithromycin, the patient was without clinical relapse.

It was thought that the patient became infected 7 years earlier during a trip to France and Switzerland because PCR results were consistent with *B. burgdorferi* **sensu lato** (*B. Afzelii*) There is no recommendation in The Guidelines for the very aggressive long-term antibiotic treatment that ultimately helped this patient. <u>This study was not referenced by The Guidelines.</u>

In a study by Oksi, the histories were presented of 3 CNS Lyme patients from whom brain biopsy was obtained for analysis.[39] Two of the 3 patients demonstrated persistent infection despite antibiotic therapy and their clinical findings are described here.

Patient #1 was a 51 year-old woman with a long history of presumed rheumatic disease who was treated with multiple courses of long term corticosteroids. Over time, her condition continued to worsen with fevers and progressive, multiple neurologic symptoms. Brain MRI demonstrated enlarged ventricles, cortical atrophy, and marked degenerative changes. The patient was seronegative for Lyme disease and CSF was without pleocytosis or *B. burgdorferi* antibodies. However, CSF cultured positive for *B. burgdorferi* **sensu lato**. The patient was treated with IV ceftriaxone for 3 weeks and partially improved. After that, treatment was changed to oral doxycycline for 8 months, but while on doxycycline therapy, she regressed. At that time, both plasma and bone marrow were Lyme PCR positive. Ceftriaxone therapy was reinstituted, but the patient died 5 weeks later. On autopsy, brain tissue was Lyme PCR was positive.

Patient #2 was a previously healthy 40 year old man who presented with seizures. Lyme IgM serologies were mildly positive, but IgG was negative. Thereafter, both serologies remained negative throughout his illness. Brain MRI showed 3 enhancing lesions. Lyme PCR and antibodies were negative in CSF and both remained negative throughout the duration of his illness despite multiple CSF analyses; however brain biopsy demonstrated positive Lyme PCR in 3 separate tissue samples. The patient was treated with ceftriaxone for 3 weeks followed by amoxicillin with probenicid for 3 weeks. After treatment, a new brain lesion appeared. The patient was re-treated with ceftriaxone for 4 weeks and azithromycin with rifampin in combination for 3 weeks. Despite treatment, 3 new brain lesions appeared. The patient was re-treated with cefixime with probenicid for 100 days with resolution of all lesions by end of treatment. Six months later, a new lesion appeared. The patient was treated with high dose doxycycline 150 mg tid for 4 months. After therapy, new brain lesions appeared and a plasma Lyme PCR was positive. The patient was treated with ceftriaxone for 100 days. After therapy, all brain lesions resolved. Plasma Lyme PCR's were negative during and at the end of treatment. A repeat brain MRI 7 months later was without lesions and a 3rd plasma Lyme PCR was negative. The patient remained clinically well.

This study documents persistent infection with *B. burgdorferi* despite extremely aggressive long term oral and intravenous antibiotic therapy. Second, it demonstrates active CNS infection despite seronegativity, CSF Lyme antibody negativity, and CSF Lyme PCR negativity. Third, it documents a fatality associated with the prior use of corticosteroids in Lyme disease. Multiple human studies have demonstrated poorer outcomes to antibiotic treatment for Lyme patients who were inadvertently treated with corticosteroids prior to antibiotic therapy.[162,163,164] Fourth, it demonstrates ultimate success in treating severe refractory Lyme disease in an immunocompetent patient, but only after particularly aggressive antibiotic therapy. Fifth, it demonstrates diminishing Lyme antibodies despite persistent infection with *B. burgdorferi*. Lastly, it illustrates partial and/or transient responses to antibiotic therapy associated with persistent infection and subsequent relapse.

Strict quality assurance was employed throughout this study. PCR was run with both positive and negative controls which remained as such throughout the experiments. *B. burgdorferi* genospecies were not specified.

There is no recommendation in The Guidelines for the very aggressive long-term antibiotic treatment that ultimately helped patient #2. <u>This study was not referenced by The Guidelines.</u>

In a study by Priem, the clinical histories were explored of 4 patients with Lyme arthritis meeting CDC surveillance case definition.[165] All patients had also initially had a positive synovial fluid Lyme PCR. After treatment with ***both*** doxycycline for 30-35 days and ceftriaxone for 14-28 days in ***all*** patients, arthritis either partially resolved and then relapsed, or did not resolve.

After treatment, in all patients synovial fluid Lyme PCR subsequently became negative, however Lyme PCR of synovial membrane remained positive after antibiotic therapy. All patients were subsequently treated with longer term IV and oral antibiotics. In 3 patients, this consisted of IV cefotaxime for 3 weeks followed by either doxycycline or minocycline for 6 weeks, and in the 4th patient, IV imipenam for 2 weeks followed by doxycycline for 6 weeks. In all cases, arthritis resolved.

Strict quality assurance was employed throughout this study with both positive and negative PCR controls which remained as such. This study demonstrates, both on clinical and laboratory grounds, persistent infection with *B. burgdorferi* despite repeated courses of antibiotic therapy recommended for the treatment of Lyme disease. It refutes the IDSA recommendation regarding "*antibiotic-refractory Lyme arthritis*" on page 1113, which states that these patients should receive symptomatic treatment. Rather, this study demonstrates that these patients can respond well to more aggressive antibiotic treatment. Second, it also documents partial and/or transient clinical responses to antibiotic therapy to be associated with persistent infection and relapse. This research was not referenced by The Guidelines.

In a study by Pícha, the clinical histories of 62 patients with Lyme disease were reviewed.[133] Of those, 19 patients had EM, 24 patients had early neurologic disease, 1 patient had early joint disease, 8 patients had late neurologic disease, and 10 patients had late joint disease. Therefore, 71% had early disease and 29% had late disease. Both before and after antibiotic therapy, patients were evaluated by a 5-primer Lyme PCR of several body fluids: CSF, plasma, urine, and synovial fluid. Before antibiotic treatment, overall PCR positivity was 58%. After antibiotic therapy overall PCR positivity decreased to 42%. This study employed strict quality control. All positive and negative controls remained as such throughout the experiments.

This study documents the persistence of *B. burgdorferi* after antibiotic therapy with newer high yield multi-primer PCR technology. It also documents a partial reduction in PCR positivity after antibiotic therapy, lending additional weight to the validity of the PCR results. This study was published *after* publication of The Guidelines.

In a study by Honegr, the clinical experiences of 18 patients with late Lyme disease were revealed.[186] Despite recommended IV antibiotics in all cases, and multiple courses of antibiotic therapy in many cases, persistence of *B. burgdorferi* by both PCR and immuno-electron microscopy was documented. The research was executed under stringent laboratory quality assurance, with both positive and negative controls throughout the procedures.

In addition to persistent infection despite antibiotic therapy, this study also documents high rates of seronegativity among patients with late Lyme disease. With initial testing, 7 out of 18 patients (39%) were Lyme ELISA negative but confirmed to have active infection by PCR and/or immuno-electron microscopy; whereas upon repeat testing, 12 out of 18 patients (67%) were Lyme ELISA negative but again confirmed to have active infection by PCR and/or immuno-electron microscopy.

This research also documents the frequency of only non-specific symptoms in late Lyme disease: 50% of patients had only non-specific symptoms at any point in their illness, and 67% of patients had only non-specific symptoms in later stages of illness. This article is covered more fully elsewhere in this testimony. <u>This study was not referenced by The Guidelines.</u>

In a study by Hulinska, 10 patients with persistent symptoms of Lyme disease despite standard antibiotic treatments are described.[166] Single courses of antibiotics were administered in 4 patients, and repeated antibiotic treatments in 6 patients. Despite this, after antibiotic therapy Lyme PCR was positive in the blood of all 10 patients, PCR was positive in the synovial fluid of 6 patients, and PCR was positive in the synovial membrane of 4 patients. *B. garinii* DNA was detected in 8 patients, *B. afzelii* in the remaining 2 patients. There was no cross amplification between species. Meticulous quality assurance was performed throughout the study with both positive and negative controls remaining as such throughout. Corroborating their PCR findings, borrelial antigens were detected in the blood of 7 of 10 patients (70%) using immuno-electron microscopy with monoclonal antibodies directed against *B. burgdorferi*.

Lyme serologies were assessed repeatedly in all patients over time, ie 4-8 times over the course of 2 years. During that time, 9 of 10 patients (90%) had at least one fully positive Lyme IgG ELISA and IgG Western Blot, while during that same timeframe, 6 of 10 patients, (60%) had at least one serologic test result that did not meet the two-tiered serologic CDC surveillance case definition.

The case histories of several patients were chronicled. Patient #2 had positive Lyme serologies and 3 positive Lyme PCR's before treatment: Blood, synovial fluid, and synovial membrane. Six months after high dose doxycycline treatment for 1 week and routine dose for another week (2 weeks total), he had recurrent arthritis with a positive Lyme PCR in synovial fluid. After 2 weeks treatment with ceftriaxone, symptoms decreased, but he relapsed 4 months later. He was then re-treated with antibiotics with benefits again, but the details of the third antibiotic treatment were not disclosed.

Patient #4 had EM after a tick bite, positive Lyme serologies, and a positive Lyme PCR in blood before treatment. After doxycycline for 3 weeks, Lyme PCR in blood was still positive, as was immuno-electron microscopy. She was re-treated with doxycycline (length not specified), but after treatment synovial fluid PCR was positive. She was then treated with IV ceftriaxone for 2 weeks and did well. Of note, IgM positivity appeared shortly after EM and remained in association with PCR positivity. When Lyme PCR became negative, so did Lyme IgM serology, correlating well with disease activity, whereas IgG levels did not.

Patient # 9 had EM confirmed by positive Lyme PCR and culture with *B. burgdorferi* sensu lato (*B. afzelii*). After doxycycline treatment, the patient still had persistently positive Lyme PCR.

This study documents the persistence of *B. burgdorferi* after both single and repeated courses of antibiotic therapy. Second, it demonstrates the persistence of *B. burgdorferi* despite antibiotics in both seropositive and seronegative patients. Third, it illustrates the correlation of IgM reactivity with disease activity, and the lack of correlation with IgG reactivity. Fourth, it demonstrates that partial and/or transient clinical responses to antibiotic therapy are associated with persistent infection and relapse. <u>This study was not referenced by The Guidelines.</u>

<u>Human Culture—Sensu Stricto</u>

A study by Oksi explored the clinical courses of 165 Lyme patients initially meeting CDC surveillance case definition who were treated with antibiotics for a median duration of 16 weeks.[167] Thirty-two patients (19%) had a relapse despite long term antibiotic therapy, 13 of whom (41%), were positive by *B. burgdorferi* culture, PCR, or both. One patient was positive by *B. burgdorferi* blood culture only, 10 patients were positive by *B. burgdorferi* plasma PCR only, and 2 patients were positive by both *B. burgdorferi* culture and plasma PCR. One of the positive blood cultures which was also PCR positive demonstrated: *B. burgdorferi* **sensu**

stricto. An additional 2 patients were asymptomatic but persistently PCR positive. All 13 patients were then re-treated with 4 to 6 weeks IV ceftriaxone, followed by oral antibiotics for 3 weeks in 3 patients, which resulted in good improvements for 9 of the 13 patients (69%).

Twelve of the 13 patients (92%) with both clinical relapse and microbiologic confirmation of persistent infection were initially seropositive. The one initially seronegative patient was both CSF culture and CSF PCR positive. After antibiotic therapy, at the time of laboratory confirmation of persistent infection by PCR and/or culture, Lyme antibody levels had diminished significantly such that only 6 of 12 patients (50%) were IgG seropositive. Patient #1 remained IgM positive but IgG seronegative.

Immediately following antibiotic therapy, only 1 of 13 patients (8%) was PCR positive (this data is mentioned in the abstract) whereas at the time of laboratory confirmation of relapse with persistent infection, 12 of 13 patients (92%) were PCR positive.

Table 8. Oksi et al.,[167] Serology, PCR, and culture status before antibiotic therapy

Patients:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
IgM Antibodies	POS	POS	POS	NEG	NEG	POS	NEG	POS	NEG	NEG	POS	NEG	NEG
IgG Antibodies	NEG	POS	POS	POS	POS	POS	POS	NEG	POS	NEG	NEG	POS	POS
Plasma PCR[a]			NEG		NEG		POS	NEG			NEG	NEG	NEG
CSF PCR[a]	POS		POS		NEG			NEG	POS	POS	POS		NEG
Biopsy PCR[a]	POS			NEG	NEG			POS					
Blood Culture[a]			NEG		NEG		POS	NEG			NEG	NEG	POS
CSF Culture[a]	POS		NEG		NEG			NEG	POS	POS	NEG		NEG
Biopsy Culture[a]	POS			NEG	NEG			NEG					

[a] *Not all patients had these tests.*

Table 9. Oksi et al.,[167] Time period until laboratory confirmation of relapse

Patients:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Abx-LCR[b]	32	130	40	86	43	22	34	44	22	4	32	0	60

[b] *Abx-LCR=Interval in weeks between finishing antibiotics and laboratory confirmation of relapse with persistent infection*

Table 10. Oksi et al.,[167] Antibiotic treatment duration

Patients:[c]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Antibiotic Duration[d]	17	16	16	14	16	16	16	47	16	28	19	17	16

[c] *Eleven of the 13 patients (85%) had been treated with <u>both</u> IV ceftriaxone and oral antibiotics.*
[d] *Total duration of antibiotics in weeks before laboratory confirmation of relapse with persistent infection*

Table 11. Oksi et al.,[167] Serology, PCR, and culture status after antibiotic therapy

Patients:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
IgM Antibodies	**POS**	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
IgG Antibodies	NEG	NEG	NEG	**POS**	**POS**	**POS**	**POS**	NEG	**POS**	NEG	NEG	NEG	**POS**
Plasma PCR	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	**POS**	NEG
CSF PCR[e]		NEG			NEG	NEG		NEG	NEG				NEG
Biopsy PCR[e]										**POS**			NEG
Blood Culture	**POS**	NEG	NEG	NEG	**POS**	NEG	NEG	NEG	NEG	NEG	NEG	NEG	**POS**

[e]*Not all patients had these tests.*

Stringent laboratory quality controls were adhered to throughout this study. Both pre- and post-PCR procedures were carried out by separate researchers in separate laboratory rooms. Hybridization of a portion of the amplified products confirmed positive PCR results. Every 6th tube of every PCR run was used as a negative control and all negative controls remained negative throughout the procedures. As such, contamination is highly unlikely. The possibility of re-infection was addressed by the authors and considered likewise improbable. The authors state, "…it is probable that reinfected patients would have developed symptoms compatible with the first stage of LB[Lyme borreliosis]. None of our patients developed erythema migrans during the follow up period, and their symptoms were similar to those before the first antibiotic treatment."

This large study of 165 well characterized patients originally meeting CDC surveillance case definition for Lyme disease reveals numerous significant findings: First, 19% of all patients experienced a clinical relapse despite long term antibiotic therapy with a median duration of 16 weeks.

Second, of those patients who relapsed, 41% demonstrated microbial evidence of persistent infection by positive PCR and/or positive culture.

Third, of the 41% in whom microbial evidence of persistent infection was demonstrated, all had received long term antibiotics of the same median duration of 16 weeks, with 85% of them having received IV ceftriaxone as well as oral antibiotics.

Fourth, this study demonstrates by both positive blood culture and positive plasma PCR, persistent infection with *B. burgdorferi* **sensu stricto** despite long term antibiotic therapy, with both IV ceftriaxone and oral antibiotic regimens totaling at least 16 weeks duration.

Fifth, at least 42% of patients had diminishing Lyme antibodies over time despite persistence of infection.

Sixth, transient clinical responses to antibiotics were associated with persistent infection, but these patients responded to re-treatment such that 9 of the 13 patients (69%), who were re-treated again with antibiotics after their clinical relapse, improved once more.

Seventh, this study documents asymptomatic infection in the 2 patients who were PCR positive but asymptomatic after antibiotic treatment.

Eighth, it documents persistent IgM reactivity but IgG negativity in late active disease.

Lastly, whereas only 8% of patients had Lyme PCR positivity immediately after antibiotic therapy, 92% became positive over time in association with their relapse of clinical disease. As such, these findings argue against fleeting genetic residue from dead borreliae as an explanation of these positive PCR findings, and rather argue in favor of resurgence of infectious burden during replication over time.

⌘Wormser has stated, "…culture of blood samples is rarely positive in patients with any objective clinical manifestation of LB other than EM…Perhaps the most fundamental limitation is that culture is far too insensitive in patients with extracutaneous manifestations of LB, which is unfortunately the group of patients who pose the greatest diagnostic confusion.[168]" ⌘Wormser and ⌘Nadelman further state, "In suspected extracutaneous Lyme borreliosis, laboratory support for the diagnosis is essential. Culture of *B burgdorferi* has been a highly insensitive diagnostic technique for these patients...[169]" Consequently, it is a reasonable expectation that this study should have been included in The Guidelines, as it helps to clarify the "diagnostic confusion" associated with chronic Lyme disease.

Despite all of the highly noteworthy findings from this research, and despite that it was performed under very meticulous quality assurance, this study was not referenced by The Guidelines. [167] Given all of the foregoing, its omission from The Guidelines was a significant error.

In a study by Haupl, a 48 year-old woman presented with vision changes diagnosed as multifocal choroiditis.[170] On review, she remembered a tick bite and rash 2 months earlier. At presentation, IgG Lyme serologies were positive, but Hauple writes, "The IgG titer rapidly decreased within a few weeks after the first antibiotic therapy, and remained negative in both the IF and ELISA evaluations, despite progression of the disease." Western Blots were consistently negative for the patient.

The patient was treated with doxycycline for 6 weeks with resolution of the choroiditis. Four weeks after stopping antibiotics, she experienced migratory arthritis of the small joints in her hands and her EKG demonstrated newly inverted P waves consistent with an ectopic atrial pacemaker which had not been present on an EKG one year earlier. She was treated with ceftriaxone for 2 weeks with resolution of both the arthritis and the rhythm disturbance.

Two months later, the choroiditis returned. CSF demonstrated normal cell counts, protein, and negative Lyme antibodies. The patient was then treated with a combination of roxithromycin and trimethoprim/sulfamethoxazole. During therapy, the patient developed severe hand pain and findings consistent with "trigger finger" of the thumb. Surgery was performed and a biopsy of the flexor retinaculum was obtained.

The authors state that transmission electron microscopy demonstrated that, "The ligament tissue was found to be heavily infiltrated by spirochetes." The ligament cultured positive for *B. burgdorferi* **LW2 (sensu stricto)**. Meticulous laboratory quality assurance standards were in use throughout this study. Cultured spirochetes from the patient's specimen were evaluated by PCR. The amplified product was hybridized using Southern blot technique. Both negative and positive controls were appropriately used throughout the procedures.

This study demonstrates persistent infection with *B. burgdorferi* **sensu stricto** despite recommended antibiotic regimens, both oral doxycycline and IV ceftriaxone. Persistent infection was confirmed by culture and PCR. It also documents diminishing Lyme antibody titers despite persistence of infection, and the absence at any time of a positive Lyme Western blot during chronic infection with *B. burgdorferi*. <u>This study was not referenced by The Guidelines.</u>

In a study by Schmidli, a 15 year old girl was bitten by a tick, did not develop EM or flu like illness, but developed Bell's palsy 3 months later.[171] Lyme serologies were positive and the patient was treated with amoxicillin/clavulanic acid for 12 days. Treatment was discontinued at day 12 due to a maculopapular rash for which she was prescribed 2 weeks of a corticosteroid. The Bell's palsy only partially resolved. An LP was negative and the patient was treated with doxycycline for 2 weeks with resolution of the Bell's palsy. Two months later, the patient had arthritis of the knee. Synovial fluid cultured positive for *B. burgdorferi*. Monoclonal antibody H5332 bound against the isolate. The majority of strains which bind H5332 are of the genospecies *B. burgdorferi* **sensu stricto**, but a minority of *B. garinii* strains will also be reactive.

This study demonstrates culture confirmed persistent infection with *B. burgdorferi*, most likely **sensu stricto**, despite both amoxicillin and doxycycline. This study was not referenced by The Guidelines.

Human Culture—Sensu Lato

In a study by Preac-Mursic, the experiences of 5 patients were chronicled.[47] Cultures from all patients were positive for *B. burgdorferi* **sensu lato** despite antibiotic therapy. Isolates were confirmed as *B. burgdorferi* **sensu lato** by monoclonal antibodies and pulsed-field gel electrophoresis. The clinical histories of the patients are presented in Table 12.

Table 12. Preac-Mursic et al.[47] Culture positive chronic *B. burgdorferi* infection despite antibiotics

Patient Clinical Timelines	Lyme Serologies IgM	Lyme Serologies IgG	Antibiotic Treatment Before Positive Culture	Positive Culture Source
#1-Radiculitis	NEG	POS	Cefotaxime 12 d	ND*
Several months after cefotaxime	NEG	NEG	None	ND
Cardiomyopathy for 7 yrs	ND	ND	None	ND
Progressive cardiac disease	NEG	NEG	None, Mitral Valve Replacement	Mitral Valve
#2-Arthritis	POS	POS	Ceftriaxone 14 d	Synovium
#3-Arthritis	NEG	POS	Ceftriaxone 14 d	Synovial Fluid
#4-Arthralgias, Headaches for 1 yr, first	NEG	NEG	None	Skin Biopsy
treatment after first positive culture	NEG	NEG	Ceftriaxone 14 d, Doxycycline 10 d	Skin Biopsy
#5-Arthralgias, after treatment, patient	NEG	NEG	Doxycycline 10 d	ND
had 1½ yrs chronic joint pain	NEG	NEG	None	Synovium

**ND=not done*

This study illustrates several important findings. First, at the time of positive *B. burgdorferi* culture, 3 out of 5 patients (60%) had either subjective symptoms only or objective signs not described by CDC surveillance case definition. For example, Patient #1 had objective findings of cardiac disease, but not atrioventricular block (A-V block). Ultimately, after 7 years of progressive cardiac disease, *B. burgdorferi* was cultured from his ailing mitral valve. Lyme borreliae have been implicated in cardiac valvular disease elsewhere,[172,173] as well as cardiomyopathy.[174,175,176]

Second, it documents initially positive Lyme serologies which diminished to negative rapidly after initial antibiotic treatment. However reversion to seronegativity did not equate with microbial cure. *B. burgdorferi* was later cultured despite prior IV cefotaxime treatment.

Third, this research demonstrates culture confirmed persistent infection with *B. burgdorferi* **sensu lato** in seronegative patients despite treatments with doxycycline, IV ceftriaxone, and IV cefotaxime. This study was not referenced by The Guidelines.

In a study by Hulinska, the evaluation of 30 Lyme disease patients using immuno-electron microscopy with monoclonal antibodies demonstrated *B. burgdorferi* in various patient samples as follows: Blood (9 patients), CSF (13 patients), and skin biopsies from EM (8 patients).[45] Ten of these 30 patients (33%) were seronegative. Three of these 30 patients (10%) had a positive culture for *B. burgdorferi*, one of whom was seronegative, and another of whom had completed over 3 months of antibiotics. Further details regarding the antibiotic treatments were not disclosed by the authors. Lyme PCR was positive for the 3 cultures and revealed *B. burgdorferi* **sensu lato**. This study demonstrates culture confirmed persistent infection with *B. burgdorferi* despite 3 months of antibiotic therapy. This study was not referenced by The Guidelines.

In a study by Hudson, a 42 year old man presented to the hospital with the following history: 2 years earlier he had a tick bite in Australia followed 16 days later by a 5 cm round, non-pruritic rash with central clearing at the tick bite site which was associated with flu like symptoms.[177] This was diagnosed as EM by his physician and treated within 2 days, so a total of 18 days after the tick bite, with doxycycline for 14 days. Two days into doxycycline, he developed a generalized rash, another focal rash of 5x10 cm, insomnia, myalgias, and arthralgias, which resolved with continued treatment, except the initial EM and new focal rash remained. Four days after discontinuation of doxycycline, insomnia returned, but also with new cognitive problems.

The EM at the site of the tick bite took about 3 months to resolve despite the doxycycline treatment. In the 18 months after doxycycline, a third rash appeared, 20 cm in diameter, in addition to the 5x10cm rash. They would both come and go in size and intensity, often disappearing completely. In addition to the recurrent skin lesions, the patient complained of chronic myalgias, cognitive problems, a feeling of "fullness in the head" rather than headache, and arthralgias. There was no history of any objective clinical features of late Lyme disease described by CDC surveillance case definition and the patient remained seronegative throughout his illness. His symptoms were severe, disabling him from working for 8 months prior to hospitalization. One week before being admitted to the hospital, the patient was re-treated with doxycycline for 1 week.

At hospital presentation, the patient had a "faint but definite" 20x30 cm lesion on his right lower chest/flank with central clearing. Biopsy demonstrated perivascular lymphocytic infiltrates. Culture of the rash resulted in *B. Burgdorferi* **sensu lato** (*B. garinii*). Cultures were confirmed by both IFA and PCR. The patient was treated with amoxicillin for 14 days followed by ceftriaxone for 15 days followed by benzathine penicillin 1.8 million units IM weekly for 12 weeks. His rash partially faded with antibiotic therapy, but continued to recur, as did his other symptoms once ceftriaxone was discontinued. He remained symptomatic during the first 3 weeks of IM penicillin, after which he was lost to follow up. It was not specified by the authors if the culture took place after the 2 courses of doxycycline or after some of the other antibiotics prescribed as well.

It is very unlikely that the positive *B. burgdorferi* culture documented in this study was due to laboratory contamination as the author states, "The immunofluorescent staining showed clumping, which is typical of initial isolates, as opposed to high passage laboratory-adapted strains. Furthermore, no isolates resembling this organism are kept in our laboratory, making it impossible for the isolate to be a laboratory contaminant." Re-infection is also highly unlikely because over the 18 months between the patient's two courses of doxycycline, the skin lesions would repeatedly come and go in the same locations. Multiple tick bites in the same exact locations repeatedly over a long period of time would be improbable. Further, he had just been re-treated again with a second course of doxycycline right up until his hospitalization and subsequent culture.

This study demonstrates culture confirmed persistent infection with *B. burgdorferi* despite antibiotic therapy, at a minimum 3 weeks total doxycycline. Second, it exhibits persistent infection in seronegative chronic Lyme disease. Third, it reveals severe, disabling, but only subjective, symptoms of chronic Lyme disease. Fourth, it documents that slow resolution of symptoms after treatment, i.e. persistence of EM for several months after completing the initial doxycycline treatment, is associated with persistent infection and relapse. <u>This study was not referenced by The Guidelines.</u>

<u>Human Culture—Genospecies Not Specified</u>

In a study by Pfister, 33 neuroborreliosis patients were assigned to receive either IV ceftriaxone or IV cefotaxime for 10 days.[178] Neurologic symptoms improved or subsided in 26 of 30 patients (87%) who were eligible for analysis of therapeutic efficacy. However, at long

term follow up after a mean of 8.1 months, 10 of 27 patients (37%) available for analysis were still symptomatic.

In 1 of 23 patients (4%) who agreed to repeat lumbar puncture at long term follow up, *B. burgdorferi* was cultured from CSF. That patient had received ceftriaxone. After treatment, her previous vigorous pleocytosis had resolved and the positive *B. burgdorferi* culture was obtained from normal appearing CSF without Lyme antibodies. *B. burgdorferi* genospecies was not determined. She also remained seronegative, but continued to have fevers, headaches, and radiculitis. There was no history of intercurrent arthropod bite or EM suggestive of re-infection.

This study was referenced by The Guidelines, but not in its full capacity. No reference was made to the fact that *B. burgdorferi* was cultured from the CSF of a seronegative but chronically symptomatic patient despite ceftriaxone treatment; nor that the CSF was Lyme antibody negative and otherwise also normal appearing; nor that 37% of patients were still persistently symptomatic at long term follow up despite prior IV third generation cephalosporin therapy.

In a study by ⌘Strle, 100 culture positive erythema migrans patients were followed after antibiotic therapy: 58 patients received azithromycin for 5 days and 42 patients received doxycycline for 14 days.[143] Despite therapy, 21 of 100 patients (21%) developed symptoms of late Lyme disease, broken down to 10 of 58 patients (17%) of the azithromycin treated group and 11 of 42 patients (26%) of the doxycycline treated group. In patients who developed late Lyme disease symptoms, 90% presented with subjective symptoms only. From one of 58 patients (1.7%) in the azithromycin group and from one of 42 patients (2.4%) of the doxycycline group, *B. burgdorferi* was cultured from normal appearing skin at the site of the previous EM.

It is unlikely that the findings in this study represent either re-infection or contamination based on the analysis previously discussed in regard to The Guidelines' evaluation of the Hunfeld and ⌘Strle study.[142] Moreover, the findings of all 3 of these similar studies corroborate each other,[142,143,144] thereby lessening the already remote possibility of re-infection or contamination for any single one study.

This study documents persistent infection despite both doxycycline and azithromycin in early Lyme disease. Second, it also illustrates that overall clinical failure rates for doxycycline were higher than those for azithromycin. Third, it documents in a prospective fashion that the large majority (90%) of patients who fail therapy for early Lyme disease go on to develop

only subjective symptoms of late Lyme disease. This study was not referenced by The Guidelines.

In another study by ⌘Strle, 107 erythema migrans patients were followed after antibiotic therapy: 55 patients received azithromycin for 5 days and 52 patients received doxycycline for 14 days.[144] Despite therapy, 28 of 107 patients (26%) developed symptoms of late Lyme disease, broken down to 10 of 55 patients (17%) of the azithromycin group and 18 of 52 patients (35%) of the doxycycline group. In patients who developed late Lyme symptoms, 88% presented with subjective symptoms only.

Before antibiotic therapy, *B. burgdorferi* was isolated from EM from 28 of 107 patients (17%) overall, of those 13 were in the doxycycline group and 15 in the azithromycin group. After therapy, *B. burgdorferi* was re-isolated from normal appearing skin in the area of prior EM from 5 of 28 patients (18%) overall, 4 of 13 previously culture positive patients (31%) treated with doxycycline and 1 of 15 previously culture positive patients (7%) treated with azithromycin. Antibiotic sensitivities of cultures before and after antibiotic therapy did not change.

As in the immediately previous study, it is unlikely that the findings in this study represent either re-infection or contamination based on the analysis previously discussed in regard to The Guideline's evaluation of the Hunfeld and ⌘Strle study.[142] Moreover, the findings of all 3 of these similar studies corroborate each other,[142,143,144] thereby lessening the already remote possibility of re-infection or contamination for any single one study.

This study documents persistent infection despite both doxycycline and azithromycin in early Lyme disease. Since antibiotic sensitivities of post-treatment isolates did not change, these positive culture findings are likely due to factors other than simply antibiotic resistance. Second, it also demonstrates that overall clinical failure rates for doxycycline were higher than those for azithromycin. Third, it reveals in a prospective fashion that the large majority (88%) of patients who fail therapy for early Lyme disease go on to develop only subjective symptoms of late Lyme disease. This study was not referenced by The Guidelines.

C. Evidence of Incongruous Clinical Definitions

Early Lyme Disease-Subjective vs. Objective Features

In a study of early Lyme disease by Trevejo, 74 patients with EM were evaluated.[179] The authors found a paucity of objective findings in early Lyme disease as detailed in Table 13.

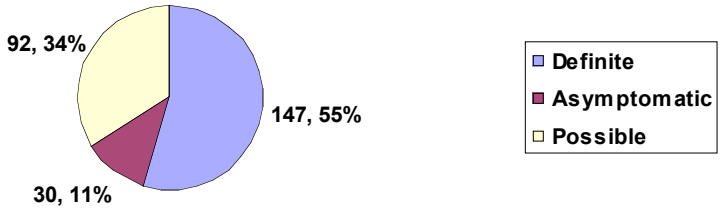
Table 13. Trevejo et al.,[179] Objective Findings vs. Subjective Symptoms in Early Lyme Disease

Objective Findings				Subjective Symptoms			
EM as Entry Criteria		Cranial Neuritis	0%	Fatigue	56.8%	Chills	35.1%
A-V block	0%	Encephalomyelitis	0%	Myalgia	43.2%	Arthralgia	35.1%
Meningitis	0%	Arthritis	11%	Headache	39.2%	(without swelling)	

All patients in this study had EM, an objective manifestation, but this was a defining entrance criterion for the study. Only 69% of Lyme cases reported to CDC from 1992-2006 had a history of EM.[180] Since EM is part of the surveillance criteria, skewing is expected with the resulting true statistic likely to be less than 69%. This is evidenced by other studies of late Lyme disease with objective features meeting CDC surveillance case definition, wherein only 22% of patients had a prior history of EM.[181] Whatever the true prevalence of EM in early Lyme, almost all agree that some significant percentage of early Lyme patients do not have EM. Since there is no valid reason to believe that early Lyme patients of same stage without EM would have higher rates of other objective features than those same stage patients with EM, the Trevejo study may be extrapolated as a valid surrogate for patients without EM as well.

In another study of early Lyme disease which spawned two journal articles by ⌘Steere, 10,936 volunteers in a Lyme vaccine study were prospectively followed for the development of early Lyme disease.[182,183] Of those, 1,917 individuals were evaluated for suspected Lyme disease. All participants had 3 sets of Lyme serologies—baseline, acute, and convalescent. Based on clinical and laboratory findings, 269 of the 1,917 suspected cases were categorized as developing "definite", "asymptomatic" or "possible Lyme disease" as defined in Figure 1.

Figure 1. ⌘Steere et al.,[182,183] Lyme Disease Categories*



**Definite Lyme Disease: (n=147) Presence of EM, cranial neuritis, meningitis, arthritis, or atrioventricular block in conjunction with at least one positive laboratory finding of: B. burgdorferi culture or PCR, or seroconversion by both IgM and IgG Western blot*

**Asymptomatic Lyme Disease: (n=30) Asymptomatic infection confirmed by Seroconversion of IgG Western blot*

**Possible Lyme Disease: (n=92) Subjective symptoms with seroconversion by either IgM Western blot, IgG Western blot, or both; or physician witnessed EM with negative Lyme serologies*

Of the patients with "definite Lyme disease", only 5 out of 147 patients (3%) had any objective features apart from EM. This corresponds well with data from Trevejo which demonstrates that only 11% of early Lyme disease patients had any objective features apart from EM.[179] Consequently, the great majority of early Lyme disease lacks other objective features.

Since the average individual is not participating in a prospective study monitoring for the development of early Lyme disease, it follows that EM in the general public would be more likely to go unnoticed. Only 22-69% of patients with Lyme disease have a history of EM.[180,181] Whether these statistics are due to EM that was overlooked, or the frank absence of EM having developed, is immaterial, for such patients are not likely to manifest other objective symptoms. This would lead to under-diagnosis if one were to mandate for the presence of objective features in order to diagnosis early Lyme disease.

In patients categorized as having "possible Lyme disease", 63 out of 92 (69%) had no history of EM or other objective signs.[182] Instead, they had developed new subjective symptoms only, and this was in conjunction with seroconversion by IgM Western blot, or IgG Western blot, or both. In a post hoc analysis, 42 of these 63 "possible Lyme disease" patients were re-tested

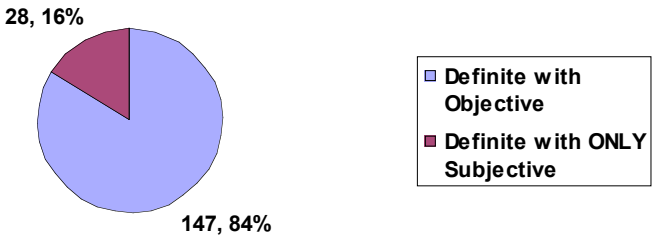
with a VlsE peptide ELISA and blood Lyme PCR.[183] If either of these tests were positive, then the authors re-classified these patients as having "definite Lyme disease." Re-classification into "definite Lyme disease" occurred for 28 of 42 patients (67%) with no history of EM or other objective symptoms.

⌘Steere excluded 21 of the original 63 "possible Lyme disease" patients (33%) from evaluation with the VlsE peptide ELISA and blood Lyme PCR by relaxing the criteria for depicting EM to include patient self-reported rashes.[183] Only physician witnessed EM, as had been described by CDC surveillance case definition, was acceptable criterion for EM in the original paper.[182] The positive predictive value of patient self-reports of possible EM is poor;[184] and EM can be challenging to accurately diagnose even for physicians.[21,185] Accordingly, in the original article ⌘Steere offered caveat in regard to the diagnosis of EM, stating, "…it may be mistaken for other dermatologic entities.[182]" Unfortunately, it is unknown as to how many of those 21 deleted patients may have also been re-classified as "definite Lyme disease" by further testing with VlsE peptide ELISA or Lyme PCR.

All 42 of the evaluated patients were offered treatment, of which 2 declined. Of the 40 treated patients, 33 got well, while 7 of 40 treated patients (18%) remained chronically symptomatic with subjective complaints. Of these treated but chronically symptomatic patients who did not manifest objective signs, 5 of 7 patients (71%) had been re-classified as having "definite Lyme disease".

This prospective study demonstrates several important points. First, it documents that "definite Lyme disease" without EM or any other objective feature is common at 16%.[182,183] This is illustrated in Figure 2.

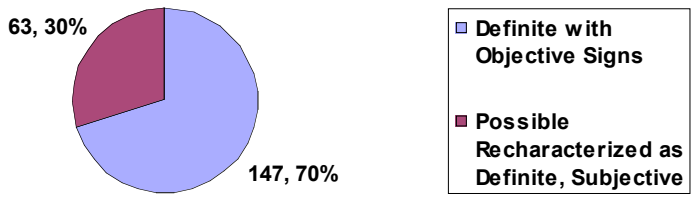
Figure 2. ⌘Steere et al.,[182,183] "Definite Lyme" with only subjective symptoms as % of total "definite Lyme"*



**Arithmetic as follows: 28/(147 + 28) = 16%*

Second, this study demonstrates that both groups with subjective symptoms only, whether "definite Lyme disease" or "possible Lyme disease", responded to antibiotic therapy in a nearly identical fashion. Resolution of newly acquired subjective symptoms after treatment with antibiotics, combined with newly acquired Lyme IgM Western blot seroreactivity, IgG Western blot seroreactivity, or both, increases the likelihood that those in the "possible Lyme disease" category did indeed have Lyme disease. Evidently the authors thought that "possible Lyme disease" had a high likelihood of representing true Lyme disease such as to warrant treatment, since patients in both categories were offered antibiotics. Figure 3 illustrates an analysis of the remaining 14 "possible Lyme disease" as well as the 21 originally deleted patients with "possible Lyme disease" by shifting all "possible Lyme disease" into "definite Lyme disease" based on this likelihood.

Figure 3. ⌘Steere et al.,[182,183] "Possible Lyme recharacterized as definite Lyme" as % of total "definite Lyme disease"*



** Arithmetic as follows: 63/(147 + 63) = 30%*
*** "Possible Lyme disease" without objective features, n = 63* [182]

Third, it calls into the question the notion of "post–Lyme disease syndrome". All these subjective-symptoms-only Lyme patients had a clinical picture identical to "post—Lyme disease syndrome" even before antibiotic treatment had begun. However, Lyme disease caused their illness; their illness responded to antibiotic therapy; and the antibiotic failure rate for their illness was the same as that which is published for early Lyme disease with objective features.[143,144]

Moreover, the rate of "asymptomatic Lyme disease," as a percentage of "definite Lyme disease" as described by ⌘Steere, was quite high at 17%, calculated from the original data as 30/(147 + 30).[182] There is also evidence from this study which further supports that

asymptomatic Lyme disease represents true infection, as vaccine efficacy was excellent against this manifestation of Lyme disease, reaching 100% by year two.[182]

Consequently, asymptomatic Lyme disease, as documented in this observational study, helps to crystallize the errors inherent to obligatively appending objective features to the diagnosis of this illness. Succinctly, if Lyme disease occurs with neither objective nor subjective features, then the notion that it does not occur in the absence of objective features becomes far less tenable.

Late Lyme Disease-Subjective vs. Objective

Most studies describing objective features in late Lyme disease are prone to selection bias because they define late Lyme disease by the *very presence* of the objective features described by CDC surveillance case definition. However, when the case definition's objective signs are not used as entrance criteria for research, then studies of late Lyme disease demonstrate that many to most patients do not manifest these objective signs. Instead, they have either subjective symptoms only, or other objective features not described by the CDC surveillance case definition.

In abundant studies of late Lyme disease with active infection demonstrated by either positive *B. burgdorferi* culture, positive Lyme PCR, or positive immuno-electron microscopy using monoclonal antibodies, many to most patients only manifest non-specific symptoms and signs not defined by CDC surveillance case definition.[47,54,150,161,167,177,186]

In one such study by Oksi, 165 Lyme patients initially meeting CDC surveillance case definition were treated with antibiotics for a median duration of 16 weeks.[167] Of this group, 32 patients (19%) relapsed after treatment. Thirteen of these relapsed patients (41%) had confirmation of persistent infection by positive *B. burgdorferi* blood culture or PCR: One patient was positive by *B. burgdorferi* blood culture only; 10 patients were positive by *B. burgdorferi* plasma PCR only; and 2 patients were positive by both *B. burgdorferi* culture and plasma PCR.

This study demonstrates that in late Lyme disease with documented persistent infection, 4 out of 13 patients (31%) did not have specific objective features described by CDC surveillance case definition. The clinical histories of these patients are outlined in Table 14. Highlighted patients had at least one objective finding described by CDC surveillance case definition. Different aspects of this study are presented in detail elsewhere in this document.

Table 14. Oksi et al.,[167] Symptoms, Signs & MRI findings of late Lyme disease

Patients:	1	2	3	4	5	6	7	8	9	10	11	12	13
Arthritis	–	–	–	–	–	–	+	–	–	–	–	–	–
Arthralgias	–	+	–	+	+	+	+	–	+	–	+	–	+
Myalgia	+	+	–	–	+	+	+	–	+	–	+	–	+
Headache	–	–	–	–	+	+	+	–	+	+	–	–	–
Dizziness	–	–	–	–	–	+	+	–	+	+	+	–	+
Meningitis	–	–	–	–	+	–	–	–	+	+	–	–	–
Radiculoneuropathy or Neuritis	–	+	+	–	–	–	–	–	–	–	–	–	–
Neuropathy	+	+	+	–	–	–	–	–	+	–	+	–	–
Carpal Tunnel	+	+	–	–	–	–	–	–	–	–	–	–	–
Diplopia	–	–	–	–	–	–	–	–	+	–	–	–	–
Seizure	–	–	–	–	–	–	–	+	–	–	–	–	–
Encephalitis	–	–	+	–	–	–	–	+	–	+	–	–	–
Hemiparesis	–	–	+	–	–	–	–	–	–	–	–	–	–
Fever	+	+	+	–	+	–	–	–	–	+	–	+	–
Hepatitis	+	–	–	–	–	–	–	–	–	–	–	+	–
Retinitis or Uveitis	–	–	–	–	–	–	–	–	–	–	–	+	–
Pleurisy or Pericarditis	–	–	–	–	–	+	–	+	–	–	–	–	–
Vasculitis-biopsy proven	+	–	–	–	–	–	–	+	–	+	–	–	–
Abnormal Brain MRI	+	ND	+	ND	ND	+	–	+	–	+	–	ND	ND

Highlighted patients have features described by CDC surveillance case definition.
History of EM excluded for this table.

In a study by Honegr,[186] the clinical histories were chronicled for 18 patients with well documented late Lyme disease. All patients had active infection greater than 3 months after treatment. The continued presence of *B. burgdorferi* was documented by immuno-electron microscopy with monoclonal antibodies, and/or Lyme PCR. This study is presented in elsewhere in this document.

Even though all patients had laboratory evidence of active infection, only 9 of 18 patients (50%) had ever had any specific objective signs of Lyme disease as described by CDC surveillance case definition. The authors stating, "The typical clinical manifestations of Lyme disease were observed in 9 patients and non-specific symptoms in another 9 patients." In this study, non-specific symptoms were comprised of headache, fatigue, myalgias, arthralgias, and low grade fevers.

Of note, 4 patients had a history of EM, one of whom later developed neuroborreliosis. In the 3 other patients with a history of EM, including one who also developed transient A-V block, EM and A-V block were their only CDC surveillance case defining specific objective signs; and they only lasted approximately 2-4 weeks. After that, despite microbiologic evidence of

continued *B. burgdorferi* infection over many months to over 5 years, they did not manifest objective signs described by the CDC surveillance case definition.

Therefore, even though the authors depict 9 out of 18 patients (50%) as having had specific symptoms of *B. burgdorferi* at some time in their disease, at the time of *late Lyme disease*, 12 out of 18 patients (67%) had only non-specific symptoms, despite laboratory evidence of persistent infection.

Table 15. Honegr et al.,[186] Clinical timeline at up to 68 months follow up

Pt[i]	Antibiotics Before Detection 1	Detection 1		Months Between Detections	Antibiotics Before Detection 2	Detection 2		CDC Case Definition Sign	Lyme ELISA	
		Method	Sample			Method	Sample		Detection 1	Detection 2[l]
#1		IEM[j]	CSF	33	Pen[c]	PCR	Plasma	EM, NB[g]	NEG	NEG
#2	Rox[a]	IEM	CSF	32	Pen, Pen	PCR	CSF	NONE	POS	NEG
#3	Dox[b]	IEM, PCR	plasma	37	Pen, Ctr,[d] Dox, Ctr	PCR	Plasma	NB	NEG	NEG
#4		IEM	CSF	16	Ctr, Azi,[e] Ctx[f]	IEM	Plasma	NONE	POS	POS
#5	Dox	IEM	CSF	10	Ctx	IEM	CSF	NONE	POS	NEG
#6	Dox	IEM	CSF, plasma	17	Ctr	IEM	Plasma	NONE	POS	POS
#7		IEM	CSF	25	Ctr	IEM	Plasma	NB	POS	NEG
#8		IEM	CSF, plasma	13	Ctr	IEM	CSF	NONE	POS	NEG
#9		IEM	CSF	18	Ctr	IEM	Plasma	NONE	POS	POS
#10		IEM	CSF	68	Pen	PCR	Plasma	NB	POS	NEG
#11	Dox	PCR	plasma	7	Pen	PCR	Plasma	EM[h]	POS	POS
#12		PCR	plasma	15	Ctr, Azi	PCR	Plasma	NONE	NEG	NEG
#13	Dox	IEM	plasma	64	Pen, Ctr, Ctx	PCR	Plasma & CSF	AV-block, EM[h]	POS	POS
#14		PCR	plasma	7	Ctr, Azi	PCR	Plasma	NB	POS	POS
#15		PCR	CSF	4	Pen	PCR	CSF	NB, GON[k]	NEG	NEG
#16		PCR	plasma	9	Pen	PCR	Plasma	NONE	NEG	NEG
#17	Dox	PCR	plasma	6	Pen	PCR	Plasma	EM[h]	NEG	NEG
#18		PCR	CSF	5	Pen, Dox	PCR	Plasma	NONE	NEG	NEG

[a] Rox=roxithromycin, [b] Dox=doxycycline, [c] Pen=IV Penicillin, [d] Ctr=IV ceftriaxone, [e] Azi=azithromycin, [f] Ctx=IV cefotaxime, [g] NB=neuroborreliosis, [h] EM and atrioventricular-block lasted only 2-4 weeks, [i] Pt=Patien,t, [j] IEM= immuno-electron microscopy, [K] GON=gonitis (knee arthritis-patient wasn't specified in the text, clarified by communication with author), [l] The text indicates that 17 patients were ELISA negative at 2nd detection whereas authors' "Table 3" indicates that 12 were ELISA negative; this was a typographical error clarified by communication with the authors; the text should have read "12" instead of "17".

This research, performed using strict laboratory quality assurance with both positive and negative controls throughout the examinations, has several noteworthy findings. First, it documents that only non-specific signs and symptoms were present in 50% of patients at any time in their Lyme disease, and that only non-specific signs and symptoms were present in 67% of patients at later stages of Lyme disease. Second, it demonstrates the persistence of *B. burgdorferi* by both PCR and immuno-electron microscopy despite recommended IV antibiotics in all cases, and multiple courses of antibiotic therapy in many cases. Third, it demonstrates both seronegative late active Lyme disease as well as diminishing Lyme antibody titers after antibiotic treatment despite the continued presence of the organism; at the time of 1st detection 7 out of 18 patients (39%) were Lyme ELISA negative whereas at the time of 2nd detection, 12 out of 18 patients (67%) were Lyme ELISA negative.

In a study by Frey, 8 patients who initially met CDC criteria for Lyme disease were persistently symptomatic despite treatment for several months to over 3 years.[161] All patients except one were treated with antibiotics upon the diagnosis of Lyme disease. After contracting Lyme disease, widespread myalgias persisted or developed in all patients, however this was not associated with objective signs. This study is presented in detail elsewhere in this document.

In 4 of the 8 patients (50%), a muscle biopsy was positive for *B. burgdorferi* by PCR, months to years after their initial Lyme disease presentation. Of this group of patients with confirmed active late Lyme disease, none of them had manifested objective symptoms. These findings are depicted in Table 16.

Table 16. Frey et al.,[161] Late Lyme disease—Clinical features *at the time of positive PCR*

Patients	Objective	Subjective	Lyme PCR Muscle
#1	None	Myalgia	NEG
#2	None	Myalgia	NEG
#3	None	Myalgia	**POS**
#4	None	Myalgia	NEG
#5	None	Myalgia	NEG
#6	None	Myalgia	**POS**
#7	None	Myalgia	**POS**
#8	None	Myalgia	**POS**

In a study by Preac-Mursic, the clinical histories of 5 late Lyme disease patients were presented.[47] All had active infection documented by positive culture. Three out of 5 patients (60%) did not have objective criteria described by CDC surveillance case definition at the time of the positive culture. Their clinical information is illustrated in Tables 17. This study is presented in detail elsewhere in this document.

Table 17. Preac-Mursic et al.,[47] Late Lyme disease—Clinical features *at the time of positive culture*

Patient	Objective-Not CDC Case Definition	Subjective	Objective-CDC Case Definition
1	cardiomyopathy	headaches, sweats, "pseudoradicular" pain	None
2	None	None	arthritis
3	None	None	arthritis
4	"skin eruption"--possible lymphocytoma	arthralgias, headaches, back pain	None
5	None	arthralgias, headaches	None

D. Evidence from Treatment Trials

On page 1101, The Guidelines state, "*There have been at least 9 randomized, prospective trials addressing the treatment of early Lyme disease in the United States. All studies used erythema migrans as the disease-defining criterion.*"

EM stage disease is the simplest, most well understood stage of the illness with the best treatment outcomes. Chronic Lyme disease is the most complex, poorly understood stage of the illness with the worst treatment outcomes. Yet the treatment of EM stage disease has been explored with at least 9 randomized prospective trials in the United States, whereas the treatment of chronic Lyme disease has been explored with only 3 NIH-funded prospective randomized controlled trials. These 3 trials were cited by The Guidelines.[187,188,189] The Fallon study had not been fully reviewed because it had not yet been published before the publication of The Guidelines.[189]

In the first study, by ⌘Klempner, the treatment group received ceftriaxone for 4 weeks followed by doxycycline at 100mg bid for 2 months.[187] Since this study evaluated a patient population with significant neurologic manifestations, it is puzzling that doxycycline at

100mg bid was used, since it does not reliably get adequate penetration into the CNS at that dose.[190] To aggravate matters, the minimum compliance rate for taking the doxycycline in this study was 75%, which would lessen CNS concentrations further.

The study design has drawn significant criticism in regard to possible selection bias and statistical errors.[191,192,193] It is beyond the scope of this testimony to perform a complete statistical analysis of the ⌘Klempner paper, however an abbreviated discourse of some problematic design components does merit attention in that they could have resulted in errors in the interpretation of data.

For example in his study, ⌘Klempner set the minimum limit for defining improvements by observed changes in the SF-36 General Health Survey higher than that set by other researchers. For example, for the physical component of the SF-36, ⌘Klempner required a change of at least 6.5 to detect a benefit to treatment. However the appropriate SF-36 benchmark is considered to be between 2.5 and 5.0 points for rheumatic diseases of comparable disability;[194] and studies have found changes of just 2.0 points to be clinically important in osteoarthritis,[195] and peripheral artery disease.[196]

For the mental health component of the SF-36, ⌘Klempner set the bar even higher, requiring a change of 7.9 to detect improvements from antibiotic therapy. To put this in perspective, it would have required that treated patients ended up exceeding the average score for the healthy population in order to detect a treatment benefit. Since it is an unreasonable expectation that antibiotic therapy could improve chronic Lyme patients to a level that is superior to that of the healthy population, these are very material statistical errors. It's like that old Henny Youngman joke, "This guy asked his doctor, 'Will I be able to play the piano after my operation?' And the doctor says 'Sure.' And the guy says, 'Funny, I couldn't do it before.'"

The study was also found not generalizable[197] as follows: Patients had previously failed an average of 3 courses of antibiotics; one third of patients had previously failed IV antibiotics for an average of 30 days; and patients had been ill for an average of 4.7 years. Logically, selection bias would skew toward failure if the same failed antibiotic were to be used a second time for the same length of treatment as that which resulted in the primary failure. Not surprisingly in light of selection bias and statistical errors, the study was terminated early due to an interim analysis which indicated that the treatment would be unlikely to provide benefit.

The Guidelines state on page 1119 that in the ⌘Klempner study,[187] "*36% of patients in the combined placebo groups had significant improvement in their SF-36 score, suggesting a*

substantial placebo effect in this patient population." Yet it goes unmentioned in The Guidelines that although at 180 days 36% had improved in the placebo group, 34% had worsened. These findings argue more in favor of variability to the underlying disease itself rather than placebo effect. The lack of a placebo response in this study is a material finding.

In the second study by Krupp, 2 of the targeted clinical outcomes of which were improvements in fatigue and cognition, the treatment group received 4 weeks of ceftriaxone.[188] Of note, 43% of patients had already failed treatment with ceftriaxone previously for a mean of 6.3 weeks, and as such, this study falls prey to the same selection bias limitations as the ⌘Klempner study.[187] This was revealed by Krupp who states, "Subgroup analyses suggest that patients who had only received oral antibiotic therapy in the past were more likely to experience improvement."

However despite these problems, the study confirmed that fatigue improved with treatment: 64% of the ceftriaxone treated group vs. 18.5% of the placebo treated group. On page 1120, The Guidelines attempt to cast doubt on these findings, stating, "*Several methodologic issues may have had a negative impact on the validity of the findings in this study* [294].[188] *One of these was the potential unmasking of patients noted by the investigators, because patients receiving ceftriaxone were more likely to guess their treatment group correctly.*"

However, comparing the percentages of patients between the treatment and placebo groups who ultimately guessed their treatment assignment correctly does *not* evaluate the effectiveness of masking. The correct way to perform this task is to compare the percentages of those who *believed* they were being treated with antibiotics in the treatment group vs. the placebo group. In the Krupp study, at the 6 month follow up those percentages were nearly equal: 69% of those treated with ceftriaxone thought they were receiving ceftriaxone vs. 68% of those taking placebo thinking they had received ceftriaxone.[188] An exaggeration of the data provides a clarifying example as follows: If 0% of both the treatment and placebo groups believed they had received antibiotic therapy, then 0% of the treatment group and 100% of the placebo group would have guessed their treatment assignment correctly. There would be no unmasking in this scenario however as there was no difference between the groups as to who believed they were on study medication. Since nobody in either group believed they were on treatment, there could be no placebo effect.

Another observation that argues against unmasking in the Krupp study is the marked difference in favorable responses to antibiotic therapy as follows: 80% of seropositive patients responded positively to antibiotics vs. only 13% of seronegative patients. Clearly, seropositive patients should not be better at guessing their treatment assignment.

However, there is considerable evidence from prospective randomized controlled trial data that the development of seropositivity in Lyme disease patients is associated with a better response to antibiotic treatment.[198] This may be due to an inverse relationship between Lyme antibody titers and the magnitude of infectious burden, which can be the result of antibodies being bound up in circulating immune complexes, documented in chronic Lyme disease patients.[25,155,167,168] Given all of the foregoing, in sum, there is no substantial evidence that unmasking was a problem in the Krupp study.

However, despite the improvements in fatigue documented by the Krupp study, the authors did not detect an improvement in cognition.[188] The authors give credible explanations for the lack of cognitive improvements, stating, "although the patients with Lyme disease showed cognitive slowing compared to healthy controls, these deficits were relatively mild, which may have contributed to the lack of a treatment effect on cognition." Not surprising, since, although an improvement in cognitive function was one of the targeted clinical outcomes, cognitive dysfunction was not part of the entrance criteria for the trial.

Nonetheless, other compelling reasons may also exist. For example, Krupp powered the trial to detect a 25% improvement in speed on the alpha-arithmetic (A-A) score, but did not disclose the baseline A-A score difference between the Lyme patients and the healthy controls. This information is crucial in determining if the targeted 25% improvement is a reasonable expectation from antibiotic therapy. Krupp's prior published work on the subject of A-A score speed differences between Lyme patients and healthy individuals demonstrates that a 25% improvement in A-A score would result in Lyme patients performing better than healthy persons.[199] Again as in the ⌘Klempner study, since it is an unreasonable expectation that antibiotic therapy could improve chronic Lyme patients to a level that is superior to that of the healthy controls; these are very material statistical errors.

In sum, Krupp's study had a 74% power for detecting its targeted change in speed on the A-A test. This means that the trial as designed had only a 74% chance of detecting, and therefore a 26% chance of missing, an already unforgivably high targeted improvement of 25%. Most comparable trials have powers of 80%-90% for <u>*reasonably*</u> targeted improvements. If the power had been increased, then the targeted improvement would have to have been even higher than the already unreasonable 25%. If the targeted improvement had been lowered to something more reasonable, then the power would have decreased even lower than the already inadequate 74%. In sum, this study was underpowered to detect a cognitive difference.

Krupp's study was however, appropriately powered at 81% to detect an improvement in fatigue. As such, its conclusion that fatigue significantly improved in the antibiotic

treatment group is valid. These improvements were sustained at 6 months follow up, which is consistent with a lasting antibiotic effect from treatment.

In the third study by Fallon, the treatment group received 10 weeks of ceftriaxone.[189] Interestingly, patients in this group had already failed previous treatment with ceftriaxone for an average of 10 weeks, equal to the entire duration of antibiotic treatment for the trial. As such, this study falls prey to the same limitations as the works of ⌘Klempner and Krupp.[187,188] However despite these restrictions, the Fallon study still demonstrated benefits to antibiotic re-treatment.[189] Cognition, fatigue, functionality, and body pain all significantly improved with antibiotic re-treatment at 12 weeks evaluation, whereas these changes did not occur in the placebo group.

Fallon's study further corroborates the findings by Krupp that fatigue improved with antibiotic re-treatment,[188] but this merits further discussion. Although present at 12 weeks, by 24 weeks evaluation, the improvements in fatigue were not sustained when evaluated by group mean differences; but when a post-hoc analysis was performed using the categorical criteria from the Krupp study, sustained benefits to fatigue were revealed in that a greater proportion of antibiotic treated patients improved vs. placebo treated patients. Such differential findings imply the presence of outliers having a deleterious effect on the relevance of the mean differences between groups. This effect becomes more problematic with smaller sample size. The Krupp study had a larger sample size than the Fallon study, and as such, was more immune to the effects of outliers. Consequently, Krupp was able to demonstrate benefits to fatigue not only by significant differences in proportions of patients, but also by mean differences.

In addition to benefits in fatigue, Fallon's study also revealed benefits to cognition from antibiotic re-treatment, whereas the Krupp did not detect a cognitive benefit.[189] Potential reasons as to why the Fallon study demonstrated benefits to antibiotic re-treatment over and above the Krupp study are as follows: First, as detailed above, the Krupp study had insufficient power to reliably detect an unreasonably high targeted treatment effect. Second, along these same lines, as opposed to the Krupp study, baseline cognitive testing revealed more considerable deficits in the chronic Lyme patients vs. healthy controls. Third, the treatment length was longer, of potential benefit when using beta-lactam antibiotics with slowly replicating organisms. Lastly, neurocognitive testing performed in the Fallon study was considerably more intensive than the A-A testing used in the Krupp study, which was essentially a battery of reaction times.

At 24 weeks follow up, the Fallon study documented relapse of the cognitive improvements gained among the treatment group during antibiotic therapy, but preservation of the benefits to body pain and functionality.[189] Recurrent cognitive dysfunction was compatible with that of the patients' original baseline Lyme disease symptoms before treatment. Similar findings of transient benefits to antibiotic therapy followed by relapse upon discontinuation have been documented in chronic Lyme disease patients who demonstrate persistent infection with *B. burgdorferi*; and these patients respond to antibiotic re-treatment.[25,39,46,156,157,158,165,167,170,177] As such, the relapse of neurologic symptoms after discontinuation of antibiotic therapy as seen in Fallon's study is most likely caused by active infection with *B. burgdorferi*.

Alternatively, the neurologic improvements followed by relapse observed in the Fallon study could be theorized as due to non-antibiotic effects of ceftriaxone, which can have neuroprotective properties.[200] Therefore, a case could be made that some of the neurologic progress could have been due to such an action. However, it fails to explain the continued improvements in the non-neurologic symptoms, for cephalosporins do not have much in the way of overt anti-inflammatory properties. Actually, some data demonstrates that both 2nd and 3rd generation cephalosporins possess pro-inflammatory properties.[201] A more likely scenario to explain the whole of the findings in the Fallon study is a partially treated infection. Evidence from at least one prospective randomized controlled trial indicates that partial responders are more likely to relapse than complete responders,[198] and there is much in the way of non-randomized studies demonstrating that partial responses are associated with relapse.[39,46,165,166,177] Although ceftriaxone penetrates blood brain barrier to a therapeutically reasonable degree for many infections, CSF to plasma ratios for patients with inflamed meninges are still only approximately 2.5%,[202] far less than penetration in non-neurologic tissues. Antibiotic action associated with superior pharmacokinetics in non-CNS tissue compartments is one mechanism that can explain the lasting improvements observed in the non-neurologic symptoms vs. the more transient response in neurologic symptoms.

In a study by Oksi, 152 patients were randomized to receive either ceftriaxone for 3 weeks followed by placebo for 100 days or ceftriaxone for 3 weeks followed by amoxicillin for 100 days.[203] Adjunctive amoxicillin was not shown to provide benefit over ceftriaxone single agent, however there were compound problems in study design and execution which make it difficult to interpret the findings of the trial.

First, the outcomes of the study were clinical impression, which lacks standardization. A more consistent and reliable evaluation could have been performed using SF-36 and/or neurocognitive testing. Second, the study population was heterogeneous, consisting of both early and late Lyme disease patients. For example, if early Lyme disease patients in the

study do not benefit from longer courses of antibiotics vs. shorter courses, this could materially cloud the detection of a long-term antibiotic treatment benefit for the late Lyme disease patients with whom the data is entangled. Third, and perhaps most importantly, the trial was run unsuccessfully, the authors stating, "The number of enrolled patients did not reach the target to have sufficient power to make a definite conclusion about the lack of efficacy of the adjunctive treatment."

On page 1096, under the heading "**OBJECTIVE**" **The Guidelines state**, "*The panel performed an extensive review of all of the randomized, controlled trials and open-label trials published in peer-reviewed, English-language journals*." As an apparent result of that review, open label antibiotic treatment trials are referenced by The Guidelines several times: Twice on page 1107 and once on page 1112. As such, The Guidelines authors apportioned suitable credit to the observed treatment benefits in open-label studies of Lyme disease patients with both neuroborreliosis and Lyme arthritis.

Historically, it has been well known that the natural course of untreated Lyme disease consists predominantly of relapsing and remitting symptoms and signs, whether subjective or objective. Consequently, by the lack of a control group, any open label trial for Lyme disease, regardless of the presence of subjective vs. objective pathology, will be subject to the same limitations inherent to the fluctuant nature of the underlying disease. As such, open-label trials studying Lyme disease patients who manifest only subjective symptoms have equal value to those evaluating patients with objective signs.

The Guidelines referenced two open label trials by Donta[50,204] in which chronic Lyme disease patients accrued benefits in association with long term oral, non beta-lactam, antibiotic treatment. On page 1120, The Guidelines authors respond to those trials, stating, "*Open-label studies for an illness that has no objective findings need to be viewed with a high degree of skepticism*." Although The Guidelines enthusiastically evaluated open label trials for neuroborreliosis and Lyme arthritis, incongruously, this does not seem to apply to chronic Lyme disease patients, who are in most need of research.

The first study by Donta was large, consisting of 277 patients seen at University of Connecticut's Lyme Disease Clinic from 1988-1993 and Boston University Medical Center from 1993 to 1995. Serologic evaluations demonstrated that overall, 29% of the patients were Lyme ELISA positive and 81% were Western blot positive; 29% had a history of tick bite, and 44% had a history of rash.

Patients were treated with oral tetracycline for 1 to 11 months (mean 4 months). Tetracycline was chosen over doxycycline, with Donta having good reasons for this, stating, "When the pharmacologic properties of doxycycline and tetracycline hydrochloride are compared, the absorption of doxycycline is sometimes better at comparable doses, but 500 mg of tetracycline three times daily achieves higher serum levels than does 100 mg of doxycycline twice daily. Because doxycycline is also highly bound to proteins (which accounts for its longer half-life), the amount of free drug available to diffuse of into tissues is less than that of tetracycline."[50] His pharmacokinetic analysis may be correct, in that some animal data demonstrates cure with tetracycline vs. failure with doxycycline.[205]

After 2 months of treatment, 33% of patients were considerably improved; after 3 months of treatment, 61% of patients were considerably improved. Ultimately with treatment, 20% of patients had complete resolution of symptoms, 70% had material improvements, and 10% failed treatment. This response is contrasted to the natural course of such patients who have been treated with placebo, where roughly equal percentages report being both better *and* worse at 180 days.[187]

The author also found that, "Patients whose symptoms had been present for >1 year had fewer cures and more treatment failures than did those patients whose symptoms had been present for <1 year. Patients with symptoms for >3 years fared poorer than did those with symptoms for either 1–3 years or <1 year. The duration of prior symptoms was also directly correlated with the time to onset of any improvement (i.e., the longer the duration of prior symptoms, the longer the time until any signs of improvement were noted." This has important implications regarding the long-term illness of patients enrolled in the 3 NIH-sponsored randomized controlled trials for re-treating chronic Lyme disease.[187,188,189] Antibiotic treatments durations in those studies may have been too short.

The second study by Donta was also large, consisting of 235 patients seen at University of Connecticut's Lyme Disease Clinic from 1992-1993 and Boston University Medical Center from 1993 to 1997. Serologic evaluations demonstrated that overall, 26% of the patients were Lyme ELISA positive and 74% were Western blot positive; 29% had a history of tick bite, and 29% had a history of rash.

Patients were treated with clarithromycin, azithromycin, or erythromycin, each in combination with hydroxychloroquine for 1 to 18 months, (mean 6 months). After 2 months of treatment, 20% of patients were markedly improved; after 3 months of treatment, 45% of patients were markedly improved.

Donta had a cogent underlying principle for the use of hydroxychloroquine, stating that since "macrolides are less active at a low pH" and there is "localization of borrelia to an acidic endosome", that macrolide activity could be "improved by alkalinization of that compartment with hydroxychloroquine.[204]" Again, his pharmacokinetic analysis may have been correct, in that for erythromycin there exists a marked disparity between *in vitro* and *in vivo* activities against *B. burgdorferi*,[206] presumably due to inactivation at lower pH. Interestingly, Donta found that all 3 macrolide derivatives performed equally well when combined with hydroxychloroquine. This finding supports his view that the well known endosome alkalinizing activity of hydroxychloroquine may have been responsible for the observation of improved *in vivo* efficacy of erythromycin. Donta further specified that patients who had been previously treated with hydroxychloroquine single agent did not respond, indicative that the response to hydroxychloroquine in this study was likely not due to its anti-inflammatory effects.

Hydroxychloroquine may have also had another mechanism of action in this study, given its *in vitro* activity against *B. burgdorferi* cystic forms.[207] Failure of previous single agent treatment with hydroxychloroquine implies that antibiotic coverage for only cystic forms may not be a viable strategy when managing patients with chronic Lyme disease.

Again the author found that longer illness resulted in worse outcomes, stating, "Patients whose symptoms had been present for more than one year had more failures (15–25% vs 6%) than patients with symptoms for less than one year. Patients with symptoms longer than 3 years fared poorer than those with symptoms for either 1–3 years or less than 1 year.[204]"

Open label trials such as these have traditionally served as starting points for the initiation of randomized controlled trials. Such studies can be designed around the salient points gleaned from the Donta trials. For example, given that *B. burgdorferi* has been demonstrated to establish both intracellular infection[52,75,76,77,78,79,80,81,82,84,170] and the formation of clinically significant spheroplasts[52,60,80,86,87,88,89,90,91,92,93,94,95,96], it makes sense to consider antibiotic treatment options apart from beta-lactam antibiotics which had been sole focus of 2 of the 3 NIH sponsored trials.[188,189] Not only do beta-lactams lack intracellular penetration, they also have limited if any effect on *B. burgdorferi* spheroplasts. A more appropriate response to Donta's good efforts may have been to endorse the need for randomized controlled studies using agents other than beta-lactams. Given the excellent safety record of long term *oral* antibiotic therapy documented in two randomized controlled studies for Lyme disease,[187,203] the risk-benefit analysis seems quite motivating for the development of randomized controlled trials using such oral agents.

In a recent open-label prospective study by Clarissou, the authors followed 100 chronic Lyme disease patients who were treated with several classes of antibiotics for 3-6 months to assess for changes in clinical status.[208] Although the authors preferred the term "tick associated poly-organic syndrome" or "TAPOS" for the studied illness, they specified that the clinical parameters were "…compatible with what had already been described as chronic Lyme disease in NEJM [12]…"[187] These patients remained chronically ill with subjective symptoms and/or objective symptoms not described by CDC surveillance case definition, despite commonly recommended antibiotic regimens for Lyme disease. Only 51% of patients had a prior positive Lyme serology at any point in time, either at inclusion or prior to the study, the authors noting, "In the early phase, serology is often negative and after a period of positivity, may become negative (loss of the antibodies, local precipitation, antigen-antibody complexes)."[208]

Signs and symptoms of the subjects were grouped into 9 categories by organ system: Endocrine, gastrointestinal, muscular, cardio-pulmonary, skin, cognitive-psychiatric, systemic, joint, and neurologic. Patients were evaluated prospectively by a standardized questionnaire at inclusion, 3 months, and 6 months, and their survey results converted into scores.

A comparison of antibiotic efficacy by agent was not the goal of the study. As such, antibiotic therapy was determined by the patients' own physicians. In decreasing order of frequency, patients were treated with 3-6 months of the following: Amoxicillin, ceftriaxone, doxycycline, clarithromycin, tinidazole, and penicillin G. As a result of this treatment, Clarissou documents that, "No case of clinical aggravation or serious adverse event was reported..."[208]

Moreover, a material benefit associated with long-term antibiotic therapy was demonstrated in this chronic Lyme disease treatment trial. They authors state, "The evaluation performed at each medical visit (month 3 and month 6) showed a decrease in the number and intensity of signs and symptoms under antibiotic treatment. The number of organ categories for signs and symptoms presented by the patients declined during the antibiotic treatment period: the percentage of patients presenting with more than four organ categories decreased from 82% at inclusion, to 39% at month 3, and to 31% at month 6.[208]" Neurologic symptoms were the most treatment refractory, corroborating the findings of Fallon and Krupp.[188,189]
See Figures 4a & 4b for clinical response to long-term antibiotic therapy.

Figure 4a. Clarissou et al.,[208] Improvements in Symptom Scores with Long Term Antibiotics

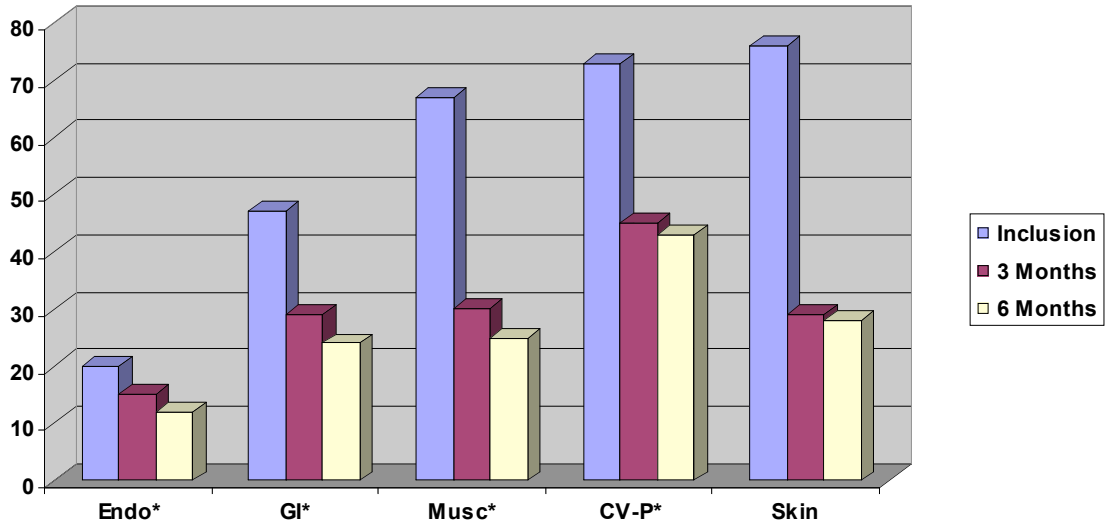
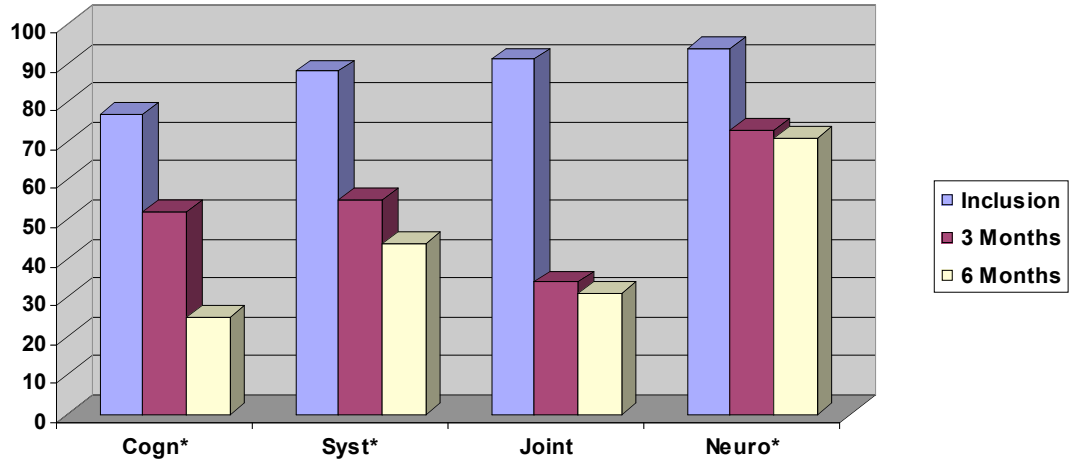


Figure 4b. Clarissou et al.,[208] Improvements in Symptom Scores with Long Term Antibiotics



***Abbreviated Headings Only**

Endo=Endocrine, GI=Gastrointestinal, Musc=Muscular, CV-P=Cardiopulmonary, Cogn= Cognitive-psychiatric, Syst=Systemic, Neuro=Neurologic

Interestingly, the authors noticed, "...around three-quarters of our study patients experienced an exacerbation of signs and symptoms during the antibiotic treatment, either early acute reactions typical of Jarisch-Herxheimer syndrome, or later subacute reactions. Exacerbation of signs could last several weeks or even several months in some patients, with the possibility of cyclic evolution."

Transient but slowly evolving and/or cyclic reactions to antibiotic therapy comprised of intensified symptoms lasting up to several months have profound implications. This implies another mechanism by which the underlying nature of chronic Lyme disease might further cloud attempts to interpret data from shorter term treatment studies. The authors came to the same conclusion, stating, "Exacerbation of signs and symptoms during antibiotherapy and the course of this exacerbation has not been well studied in the chronic forms of Lyme disease. These exacerbation phenomena may impede the evaluation of clinical improvement and could be partly responsible for the negative results of antibiotic treatment in chronic Lyme disease reported..."

This large open-label prospective study found material benefits to the long term antibiotic treatment of chronic Lyme disease patients. The authors state, "This treatment, even if it did not cure all the patients, led to improvement of quality of life, with reinsertion in the family life and often return to work." Randomized controlled trials can easily be designed using the important observations from this study.

Treatment recommendations for chronic Lyme disease must be based on sound risk-benefit analyses. "First, do no harm" is paramount. However, harm may come in many forms, including the withholding of antibiotic therapy from chronic Lyme disease patients.[162]

On the benefit side of the equation, antibiotic re-treatment for this patient population has been demonstrated to be helpful. Of the NIH-sponsored randomized controlled trials, 2 out of the 3 showed some sustained benefits from antibiotic re-treatment.[188,189] Moreover, several open label trials have all demonstrated benefits associated with long term antibiotic treatment of chronic Lyme disease patients.[50,204,208]

On the risk side of the equation, some studies have documented substantial numbers of adverse events associated with prolonged IV antibiotic therapy,[189] whereas others have documented an excellent safety record with both long term oral and IV antibiotics.[208] Whichever statistic more accurately reflects reality; it is generally accepted that prolonged IV antibiotic therapy carries with it more risk than prolonged oral antibiotic therapy and as such, its use must be evaluated in the context of an appropriate risk-benefit analysis.

Without antibiotic therapy, it is well known that these patients are persistently ill, with documented severe functional limitations which can be disabling.[187,189] Further, although post-mortem determinations for cause of death can be challenging, there have been many reported fatalities where *B. burgdorferi* infection was considered the likely cause by reasonable medical probability.[31,32,39,55,112,113,152,155,174,209,210,211,212,213,214,215,216,217,218,219,220,221,222]

Long term IV antibiotic therapy has a mixed risk-benefit analysis,[189,208] and as such must be approached individually until studies provide further clarification. However this therapy should not be dismissed out of hand, for to put things in perspective, other medications with far worse risk benefit equations are routinely used in medicine. For example, infliximab (Remicade) causes lymphoma[223] tuberculosis[224] and death[225] and is associated with a 99.7% relapse rate upon discontinuation after 3 years of continuous use by IV infusion.[226]

Trials focusing largely on long term oral antibiotic therapy have demonstrated an excellent safety record.[50,203,204,208] So it would seem that long term oral antibiotic therapy can be provided safely and that such therapy has merit for this population. From a risk-benefit analysis, even though adequate prospective randomized controlled trials have not yet been performed, it makes sense to offer such treatment to chronic Lyme disease patients based upon: Excellent results in open label trials; excellent safety records; and poor quality of life in untreated patients with chronic Lyme disease.

III. Conclusion

Contested Recommendation

> *Pages 1120-21. "To date, there is no convincing biologic evidence for the existence of symptomatic chronic B. burgdorferi infection among patients after receipt of recommended treatment regimens for Lyme disease. Antibiotic therapy has not proven to be useful and is not recommended for patients with chronic (6 months) subjective symptoms after administration of recommended treatment regimens for Lyme disease (E-I)."*

This recommendation is contested in 2 parts as follows:

a) *"To date, there is no convincing biologic evidence for the existence of symptomatic chronic B. burgdorferi infection among patients after receipt of recommended treatment regimens for Lyme disease."*

There has been considerable evidence presented within the body of this testimony which documents by culture, PCR, and immuno-pathologic means, the continued presence of *B. burgdorferi* infection in chronic Lyme disease patients who are chronically and/or recurrently symptomatic after the receipt of recommended treatment regimens for Lyme disease.

b) *"Antibiotic therapy has not proven to be useful and is not recommended for patients with chronic (6 months) subjective symptoms after administration of recommended treatment regimens for Lyme disease (E-I)."*

There has been considerable evidence presented within the body of this testimony which documents, in both randomized controlled trials and open label trials, benefits to antibiotic therapy for chronic Lyme disease patients. Two out of the 3 NIH-sponsored randomized controlled trials showed both sustained and unsustained benefits from antibiotic re-treatment. Moreover, several open label trials have all demonstrated substantial benefits as well as safety associated with prolonged antibiotic treatment.

It is suggested that the contested recommendation be removed and replaced with the following:

Revised Recommendation

"There is convincing biologic evidence for the existence of symptomatic chronic *B. burgdorferi* infection among patients after the receipt of recommended antibiotic treatment regimens for Lyme disease. Antibiotic therapy has proven to be useful and is recommended for chronic Lyme disease patients, i.e. patients with chronic (6 months) subjective symptoms after administration of recommended treatment regimens for Lyme disease (A-I). However, the best antibiotic treatments have not been established for this manifestation of Lyme disease. Prolonged intravenous antibiotic therapy carries additional risks and should be evaluated on a case by case basis. Prolonged oral antibiotic therapy has had very good results in open label trials and has demonstrated an excellent safety record, but randomized controlled trials for such therapies are lacking. Given the increased risks to prolonged intravenous antibiotic therapy, this treatment is recommended for chronic Lyme disease patients who remain seriously ill despite prior antibiotic treatment with at least 6 weeks of intravenous antibiotics and/or 3 months oral antibiotics; and where the benefits outweigh the risks (A-I). Prolonged oral antibiotic therapy is recommended for chronic Lyme disease patients (A-II).

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