Infectious Diseases Diagnosis & Treatment
Diederich J, et al. Infect Dis Diag Treat 7: 268.
www.doi.org/ 10.29011/2577- 1515.100268
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Research Article

Classical Versus Botanical Antibiotics in Lyme Borreliosis: An *In-vivo*, LTT-Controlled Study

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Citation: Diederich J, Szczerba K, Foltin V (2024) Classical Versus Botanical Antibiotics in Lyme Borreliosis: An In-vivo, LTT-Controlled Study. Infect Dis Diag Treat 8: 268. DOI: 10.29011/2577-1515.100268.

Received Date: 16 October 2024; **Accepted Date:** 21 November 2024; **Published Date:** 26 November 2024

Abstract

Purpose: Lyme disease is a common zoonosis caused by the bacterium Borrelia burgdorferi sensu lato. The disease is associated with potentially serious clinical manifestations, such as cutaneous, neurological, psychiatric, rheumatoid, cardiac and ophthalmologic symptoms. Patients are typically treated with classic antibiotics. The aim of this study was to compare the efficacy of classic and botanical antibiotics in the reduction of Lyme borreliosis activity. **Patients and methods:** A retrospective analysis of data from 452 patients with suspected Lyme disease was conducted. Laboratory screening entailed the lymphocyte transformation test (LTT) as an indicator of disease activity and serological assessment of *Borrelia* antibodies. For 116 patients, follow-up data was available, of which 58 had been treated with classic antibiotics, 48 with botanical antibiotics, and 10 had not received antibiotic treatment. The LTT stimulation index (SI) was compared between treatment groups and time points. **Results:** 47.6% of all patients had an LTT-SI > 3, representing a strong lymphocyte activity. Only a fraction of these patients (20,4%) also had detectable antibodies against *Borrelia*. In patients without treatment, LTT-SI significantly increased over time ($p < 0.05$ for all LTT values), while in both antibiotic treatment groups, LTT-SI significantly decreased over time ($p < 0.001$ for all measurements in both groups). There were no significant differences in terms of LTT-SI between treatment groups. **Conclusion:** Treatment of Lyme borreliosis patients with classic or botanical antibiotics results in a diminished immune response and decreased disease activity. Due to its high prevalence and the importance of an early detection of Lyme disease for patient outcomes, diagnosis based on the LTT-SI is recommendable and more reliable than assessment of *Borrelia* antibodies.

Abbreviations: LTT: lymphocyte transformation test; OspC: outer surface protein C; SI: stimulation index.

Introduction

Vector-borne diseases have a high prevalence and co-transmission or co-infection is frequently observed. Lyme disease, caused by the bacterium *Borrelia burgdorferi sensu lato*, is the most common zoonosis. *Borrelia burgdorferi sensu lato* can be detected in around 20% of adult ticks in Europe, depending on the season, region and detection method [1]. Lyme disease often remains unnoticed or undiagnosed and can have many different clinical manifestations, including cutaneous, neurological, psychiatric, rheumatological, cardiac and ophthalmological symptoms [2]. Moreover, there is evidence that the infection is involved in the development of autoimmune diseases such as systemic lupus erythematosus, scleroderma, dermatomyositis and systemic sclerosis [3]. Chronic infections consume a lot of energy (up to 60% more than the daily "normal"), thus leading to a state of fatigue, eventually to a chronic fatigue syndrome [4]. Some patients with Lyme disease report persistent symptoms after treatment. Spirochetes can adapt in a given environment and modify their gene expression accordingly, as they do under antibiotic pressure, leading to persisting subpopulations. Persister cells can adopt different sizes and shapes, form round bodies, L-form bacteria, microcolonies and biofilms, eventually escaping identification by the immune system and being responsible for antibiotic tolerance [5,6].

Treatment typically entails classic antibiotics such as doxycycline. However, doxycycline may induce the formation of persister cells [7]. Therefore, alternative antibiotics that are suitable for patients presenting chronic symptoms and those who have previously received unsuccessful treatment, may be indicated. Minocycline is a classic antibiotic that exhibits certain additional features such as its intraneuronal penetration and anti-inflammatory effect [8]. Tinidazol, another classic antibiotic, has a high activity (> 90%) against persister cells [9].

In addition to these classic antibiotic agents, botanical substances exhibiting antibiotic activity may be used in the treatment of Lyme disease. Feng et al. investigated the *in vitro*-activity of botanical substances against *Borrelia* and observed a high activity for *Cryptolepis sanguinolenta*, *Juglans nigra*, *Polygonum cuspidatum*, *Uncaria tomentosa*, *Artemisia annua*, *Cistus creticus*, and *Scutellaria baicalensis*. All these ingredients have a proven activity against spirochetes [10] or the capacity to reduce the activity of the transcription factor NF-kB [11]. For other substances, no activity could be observed [12,13].

The limited suitability of serological markers for the diagnosis of Lyme borreliosis has been described [14-16]. The lymphocyte transformation test (LTT) is an alternative diagnostic tool to detect Lyme borreliosis and to evaluate disease activity [17]. The LTT tests the reaction to *Borrelia afzelli* (LTT1), *Borrelia garinii* (LTT2), *Borrelia sensu stricto* (LTT3), and *Borrelia* outer surface protein C (OspC, LTT4). It results in a stimulation index (SI) that reflects the activity of lymphocytes, thereby giving an indication for immune reactions and disease activity. The higher the LTT-SI, the higher the the higher the disease activity.

The aim of this study was to compare the efficacy of classic and botanical antibiotics in the reduction of Lyme borreliosis activity. A second outcome was the suitability of the LTT in comparison with the detection of *Borrelia* antibodies as a diagnostic tool for Lyme disease. The research question to be answered was: Are classic and botanical antibiotics both effective in reducing the activity of Lyme borreliosis? If so, is one treatment more effective than the other?"

Materials and Methods

Patient cohort and data collection

The data analyzed in this study was collected between November 2020 and April 2024 from a cohort of patients who were clinically suspected of having Lyme disease. Data was extracted from patient files and included basic demographic information, results of laboratory tests such as serological markers (IgM and IgG antibodies), LTT, the type of intervention and the time elapsed between the before and after treatment measurements. After a thorough explanation, patients chose between the antibiotic treatment options.

Antibiotic treatment

The classic antibiotic prescription entailed Minocycline and Tinidazol. Patients were instructed to take 50 mg Minocycline once daily for five days, stepping up by 50 mg every five days until they reached a dose of 100 mg twice daily, which was maintained for another 20 days. During these last 20 days of Minocycline treatment, patients were also prescribed 500 mg Tinidazole per day, to be taken at nighttime. The overall duration of the treatment was 35 days. This treatment is consistent with official guidelines [18].

If a botanical treatment was preferred, a ready-to-use herbal tincture with activity against spirochetes, was administered. It contained Astragalus membranaceus, Smilax ornate, Polygonum cuspidatum, Cryptolepis sanguinolenta, Rosmarinus officinalis, Juglans nigra, Artemisia annua, Scutellaria baicalensis, Cistus incanus. Adult patients were instructed to take six pipettes (6 ml) once daily orally for six weeks, children received an adapted dose (1/ml pipette per 10kg bodyweight). For adult patients, we suggested to take a tincture containing ethanol, for children, we recommended a tincture containing glycerin.

Laboratory parameters

Disease activity was determined using the LTT as an initial

screening tool and to monitor changes in disease activity after the treatment period. The specifics of the LTT have been described elsewhere [19]. *Borrelia* antibodies were detected using CLIA (ChemiLuminescent ImmunoAssay).

Ethics approval

None of the patients was treated for the purpose of this study, and all patients participating gave their written informed consent. Due to the retrospective character of the study, ethics approval was not mandatory.

Statistical data analysis

Given the relatively small sample sizes and the lack of a normal distribution of the data, the nonparametric Wilcoxon signed-rank test method was employed to compare the median LTT-SI values between time points and Wilcoxon rank-sum test to compare the differences in values between the two treatments groups.

Results

Patient characteristics

Initial laboratory tests to determine the LTT-SI and *Borrelia* antibodies were performed in 452 patients. Of these, 265 (59%) were women and 187 (41%) were men. The average age was 45 ± 15 years (range: 8 – 90 years). Follow-up tests were available for 116 patients, of which 58 had received classic antibiotics, 48 patients botanical antibiotics, and 10 patients had received no antibiotic treatment.

LTT-SI of all participants

452 patients underwent an initial LTT-screening, we evaluated serological results in 347 of those patients. 215 patients had an SI > 3, 20,4% of the subgroup presented antibodies. 79 patients exhibited an SI between 2 and 3, 30,6% having detectable *Borrelia* antibodies. For 158 patients, the LTT-SI was below 2, with 25% exhibiting detectable *Borrelia* antibodies. Either IgM or IgG antibodies were considered positive.

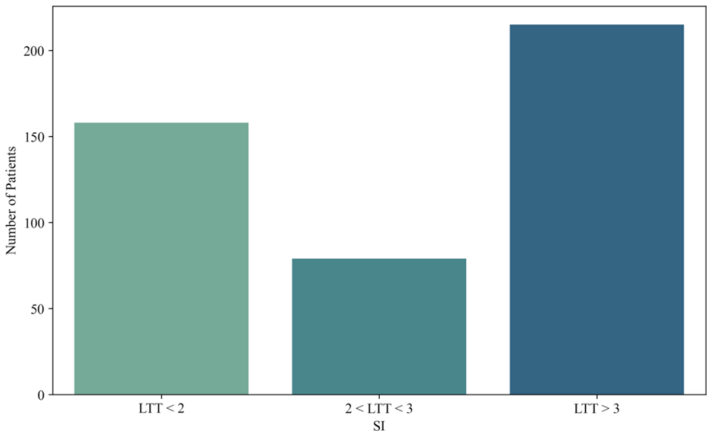

Figure 1: Distribution of the LTT-SI amongst all tested patients (n = 452).

LTT-SI development over time

The time interval between the two measurements in the control group was 69.0 (40.3 – 97.7) weeks. As shown in Figure 3, the LTT-SI of all four LTT variants increased significantly between the two measurements in patients who did not receive antibiotic treatment (LTT1: $p < 0.001$; LTT2: $p < 0.05$; LTT3: $p < 0.001$, LTT4: $p < 0.001$). At baseline, 2 (20 %) control patients were positive (LTT-SI>3), while 80 % (n = 8) were positive at the follow-up measurement.

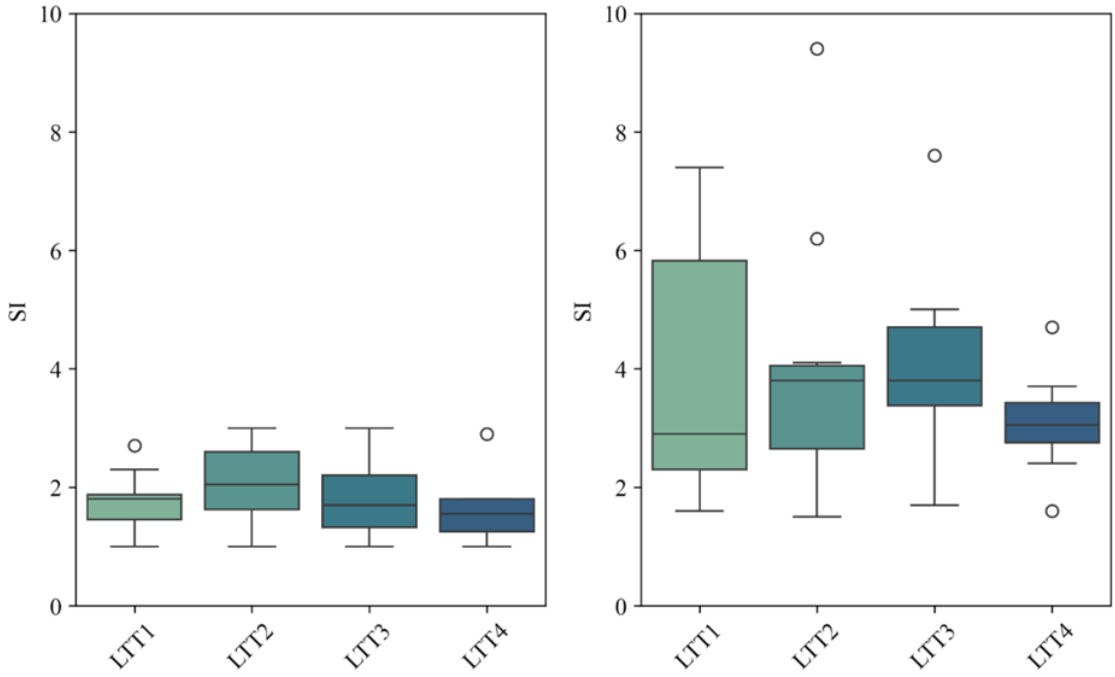

Figure 2: Changes in LTT in the control group (SI: stimulation index). Left: baseline measurement, right: follow-up measurement.

In the group receiving the classic antibiotic treatment (n = 58), the time interval between the two measurements amounted to 38.9 (31.8 – 46.1) weeks. As shown in Figure 3, the LTT significantly decreased after completion of a treatment regimen with classic antibiotics ($p < 0.001$ for all LTT measurements).

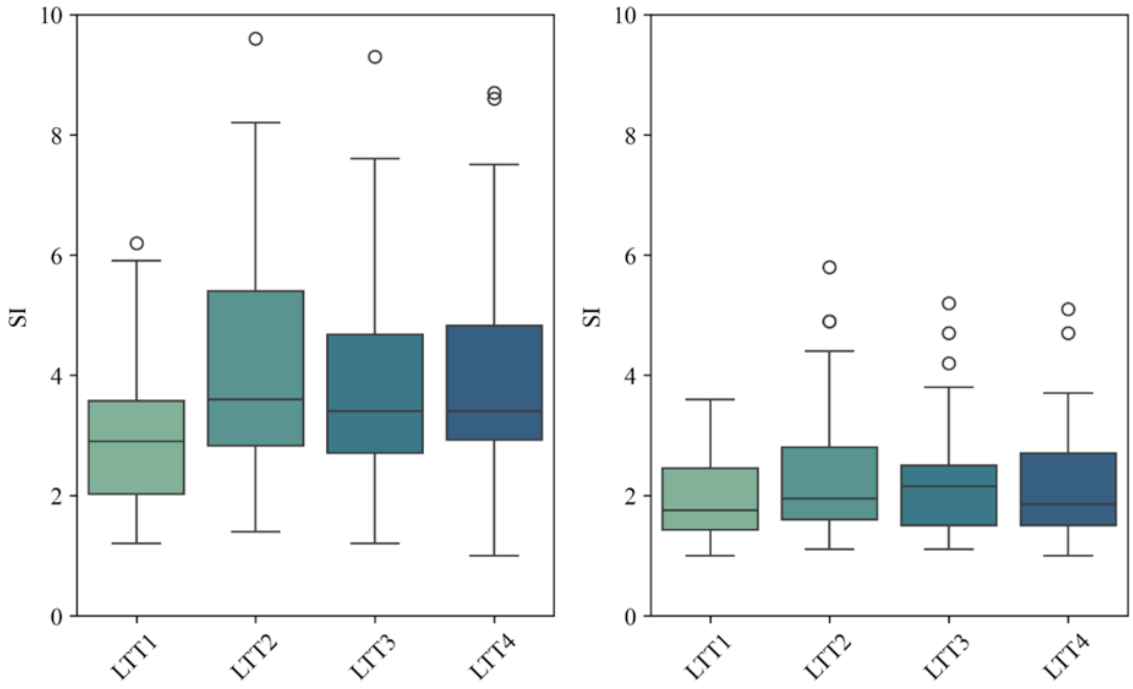

Figure 3: Changes in LTT in the classic antibiotic group (SI: stimulation index). Left: baseline measurement, right: follow-up measurement.

In the botanical antibiotic group, the time interval between the baseline and follow-up measurements was 39.0 (30.4 – 47.6) weeks. As shown in Figure 4, the LTT significantly decreased after completion of a treatment regimen with botanical antibiotics ($p < 0.001$ for all LTT measurements).

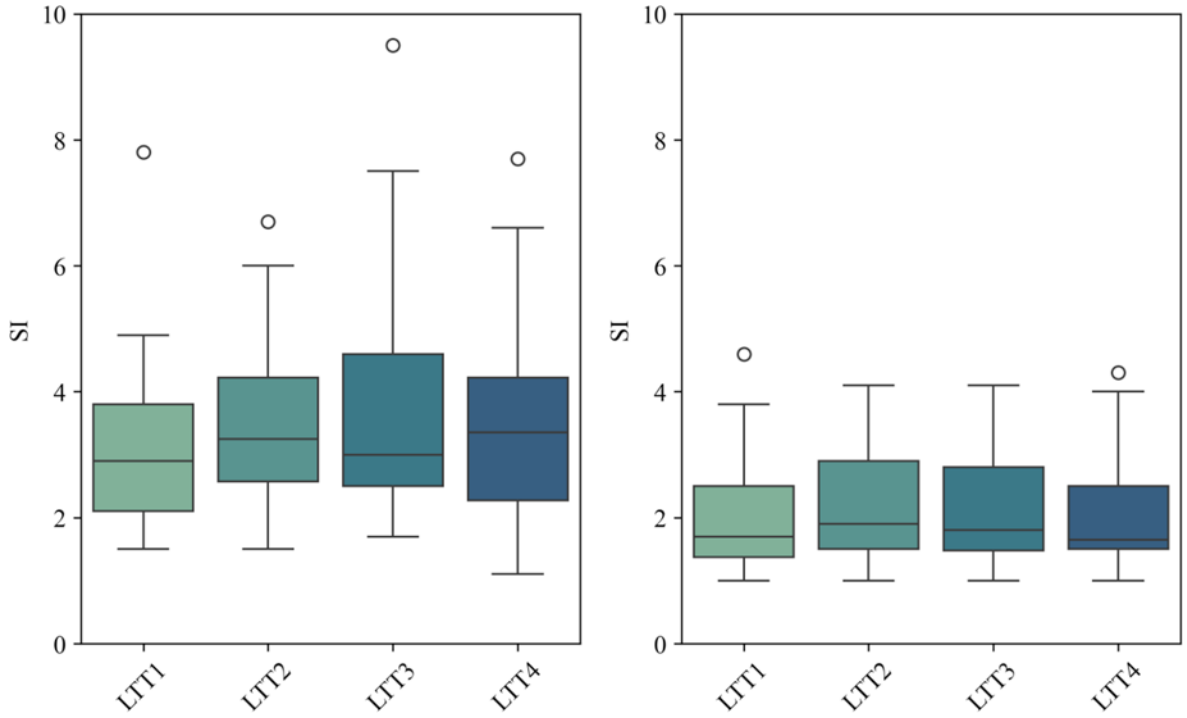

Figure 4: Changes in LTT in the botanical antibiotic group (SI: stimulation index). Left: baseline measurement, right: follow-up measurement.

Comparison of classic and botanical antibiotic treatments

The LTT results at follow-up were compared between patients who had received the classic antibiotic treatments and those treated with botanical antibiotics to determine whether one treatment regimen was superior to the other. There were no statistically significant differences between both treatment regimen in terms of the LTT-SI (LTT1: $p = 0.61$; LTT2: $p = 0.25$; LTT3: $p = 0.89$, LTT4: $p = 0.42$).

Discussion

The main finding of this study is that both classic and botanical antibiotic treatment significantly reduce disease activity in patients with Lyme borreliosis. We could not observe a difference in efficacy between both treaments, while disease activity increases in patients without antibiotic treatment. A second observation of this study is that the LTT-SI serves as a relevant diagnostic marker for Lyme disease, while the detection of *Borrelia* antibodies does not. However, when only considering the antibody prevalence, Lyme disease reaches public health relevance, as data from a review paper published in 2022 estimate that more than 14% of the world's population might be infected with *Borrelia* [20].

These observations are in line with the results of previous studies. Feng et al. report that components of a herbal tincture are able to diminish Lyme disease activity *in vitro* and even eradicate *Borrelia burgdorferi* in the stationary phase. [14] Moreover, the limited specificity of *Borrelia* antibody detection in the diagnosis of Lyme disease has been reported. [6] However, several open questions remain. As Feng at al. pointed out, it is unclear which component of the botanical antibiotic acts against *Borrelia* and against which morphological form of the bacterium (spirochetes, biofilm-like, round bodies). It has been demonstrated that antibiotics act with differing efficacy against the morphological forms of *Borrelia,* which must be considered in prescribing such tinctures. Based on the finding of the present study that there was no significant difference between the classic and botanical antibiotic, the question arises whether the botanical tincture could have any advantage over the classic antibiotic. The patients in the present study were allowed to choose the antibiotic for themselves after they had been informed about the specifics of each antibiotic. Some patients consider a herbal treatment more natural and therefore select it over the classic antibiotic. In addition, the herbal antibiotic may not pose the danger of resistance as observed with classic antibiotics.

Future studies must elucidate whether from a clinical standpoint both types of antibiotics are indeed comparable. It is possible that they exert different effects on the distinct clinical symptoms.

Nonetheless, the applicability of the results is limited by certain factors that should be addressed as follows. First, the evaluation of the course of symptoms after initiation of the antibiotic treatment was not evaluated in this retrospective study, as the primary outcome was the disease activity based on the LTT-SI and as the clinical manifestations are often biased by other comorbidities. It is of interest to evaluate changes in the symptom burden to comprehensively compare both types of antibiotic treatment. Most patients with Lyme disease have multiple symptoms and are more susceptible to other infections and diseases, which may require distinct treatment approaches in addition to antibiotic treatment. It must be acknowledged, however, that, in this cohort, the antibiotic treatment against Lyme borreliosis caused many patients to be entirely free of symptoms at the end of the treatment period. The study was conducted partly during the Covid-19 pandemic, immunological effects due to possible infection or vaccination have not been taken into consideration, as well as the multiple immunological impacts dental-related triggers can have [21]. Another limitation of this study is the small size of the control group. Moreover, the average time between the baseline assessment and the follow-up assessment was much longer in the control group than in the treatment group, which may skew the results, as a longer disease period may also exacerbate disease activity. Furthermore, new infections can have occurred during the time lapse. Nonetheless, antibiotic treatment caused a significant reduction in disease activity even after the comparatively short follow-up period, indicating that both antibiotic treatments are highly effective.

Considering the possible side effects of antibiotics, the administration of herbal substances represents an equivalent alternative. Antibody determinations appear to be obsolete. Further studies should examine the effect on the clinical manifestations.

As we are confronted to major public health challenges such as antibiotic resistance, global warming and the related shift of occurrence of vector borne diseases, and physical expositions (e.g. to metals), we need to adapt our response [22,23].

Conclusion

Every second patient tested positive for Lyme borreliosis in the LTT. We did not observe a statistically significant difference in the effectiveness on LTT-SI between the classic and the botanical treatment, both treatments demonstrated beneficial effects. Hence, treatment of Lyme borreliosis patients with classic or botanical antibiotics results in a decreased disease activity. Botanical antibiotics are an alternative to the classic antibiotics. Due to the importance of an early detection of Lyme disease for patient outcomes, diagnosis based on the LTT-SI is recommendable and more reliable than assessment of *Borrelia* antibodies.

Acknowledgments

Author Contributions: All authors contributed to the work reported, including the conception, design, and execution of the study, as well as the acquisition, analysis, and interpretation of data. All authors participated in drafting, revising, or critically reviewing this article and gave final approval of the version to be published.

Funding: No funding was received for this study.

Disclosure: The authors report no conflicts of interest in this work.

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