Persistent symptoms attributed to Lyme disease and their antibiotic treatment

Results from the PLEASE study

- Anneleen Berende -
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BACKGROUND

Lyme borreliosis, or Lyme disease, is the most common tick-borne infection in North-America, Europe and Northern Asia. The name comes from the town Old Lyme in Connecticut, USA, where the full syndrome now known as Lyme disease was first recognized in the mid 1970s. Lyme borreliosis is caused by a helically-coiled Gram-negative bacterium, the spirochete *Borrelia burgdorferi* sensu lato. Although more than ten species of *Borrelia* exist, in North America the only species known to cause human disease is *Borrelia burgdorferi* sensu stricto (hereafter referred to as *B. burgdorferi*). In Europe, there are at least six pathogenic species: *B. afzelii*, *B. garinii*, *B. burgdorferi*, *B. spielmanii*, *B. bavariensis*, and *B. myamotoi*. At least three other species (*B. bissettii*, *B. lusitaniae*, and *B. valaisiana*) have very occasionally been detected in patients, but these are not recognized as important pathogens. *B. afzelii* and *B. garinii* infections account for most Lyme borreliosis cases in Europe, whereas *B. garinii* is predominant in Asia.

*Borrelia* is transmitted by ticks of the Ixodes complex, with *I. ricinus* and *I. persulcatus* being the primary vectors in Europe and Asia. *I. scapularis* and *I. pacificus* are the most important vectors in North America. At any stage (larva, nymph, adult) of their two-year lifespan, ticks feed only once. At every meal they can acquire the infection, and upon the next feeding they can transmit *Borrelia* by injection of bacteria-containing saliva into the skin. Transmission is achieved primarily by nymphs, as they are small and consequently not rapidly noticed. This is important, as the chance of transmission of *Borrelia* to a mammalian host increases when the tick is attached for a longer period of time. Ticks feed on a large range of animals, and although many animals do not act as a reservoir for *Borrelia*, they are essential as they supply nutrients for the ticks. In Europe, rodents such as mice, voles, shrews, hares and several birds are significant reservoirs. In North-America, mostly rodents and birds are reservoirs.

EPIDEMIOLOGY

Lyme borreliosis is prevalent in Europe between 35°N and 60°N, and in North America between 30°N and 55°N. In Africa and Asia, it is only prevalent in the (upper) north. When the altitude raises, the prevalence rate of *Borrelia burgdorferi* in *I. ricinus* drops. Borreliosis
can affect all ages, but it has a bimodal distribution with a peak in children of 5 to 9 years and adults of 50 to 64 years. In most countries, the incidence of Lyme borreliosis has a peak in May up to July. During the past decades, the incidence has been increasing. In the USA, the number of reported borreliosis cases doubled from 9,908 to 19,931 cases. Incidence rates vary per state and per year with the majority of cases in New England and the mid-Atlantic states, where incidences since 2006 have further increased from 26 cases per 100,000 persons to up to 86 per 100,000 persons in 2016.

In the Netherlands, the incidence of the most common form of borreliosis, erythema migrans (EM), has risen from 39 per 100,000 in 1994 to 134 per 100,000 persons in 2009. In 2017, approximately 25,500 cases of EM were reported by Dutch general practitioners. The areas with the highest risk were Zuid-Friesland, Achterhoek, Drenthe, Veluwe, Utrechtse Heuvelrug and the coastal dunes, including the Wadden islands (Figure 1).

From 1994 to 2009 the number of reported tick bites has tripled: from 33,000 in 1994 to 93,000 in 2009. Long-term trend analyses have provided circumstantial evidence for an increase in the risk of acquiring a bite of a tick infected by Borrelia burgdorferi. An increase in the total number of Borrelia-infected ticks was not due to an increase in infection rate of ticks, but due to an elongation of the annual tick questing season next to extension of the surface area of tick-suitable habitats, most likely because of climatic changes.
CLINICAL MANIFESTATIONS

Clinical manifestations of Lyme borreliosis can be divided into three stages: early localized, early disseminated, and late disseminated Lyme disease. The most common and well-known form of borreliosis is erythema migrans (EM), an early localized borreliosis manifestation. The EM appears on the skin several days to weeks after the tick bite (range, 3 to 32 days), usually at the same location. Initially, it manifests as a small red papule or macula that subsequently enlarges, often with central clearing. Most common sites of the EM are the legs and groin. Non-specific symptoms and signs such as fatigue, headache, arthralgia, myalgia and fever can accompany the EM. In a study in the Netherlands, the risk of developing EM after a tick bite was 2.6% (95%CI: 1.1%–5.0%). This risk increases when the tick was substantially engorged or was attached for a long time. A far more rare form of early localized borreliosis is the borrelial lymphocytoma, typically located at the ear lobe or nipple.

Early disseminated borreliosis can occur within days to weeks after infection. The bacteria disseminate from the skin to other organs such as joints, causing arthritis, and the peripheral and/or central nervous system (CNS). B. burgdorferi can also spread to other skin sites, causing multiple erythema migrans lesions. Lyme arthritis is usually oligoarticular, and mostly occurs in the knee joint. Infection of the nervous system (neuroborreliosis) includes lymphocytic meningitis, cranial neuritis (most often presenting as facial nerve palsy), and painful meningoradiculitis or radiculoneuritis (also known as Bannwarth syndrome). Neuroborreliosis occurs in up to 10 to 20% of non-treated patients. In rare cases, the heart can also be infected, causing (myo)carditis.

Acrodermatitis chronica atrophicans (ACA) is an example of late disseminated borreliosis. It is characterized by a purple skin lesion that may be noted many years after an infected tick bite, and does not disappear spontaneously. Usually, it is located on the feet or hands. At later stages, the skin can become thin and wrinkled. ACA may be accompanied by sensory peripheral polyneuropathy. Other forms of late disseminated borreliosis are rare, and include chronic arthritis and chronic neuroborreliosis. Chronic neuroborreliosis may present as a long existing encephalitis, encephalomyelitis, meningo-encephalitis, or chronic radiculomyelitis.
Different species of *Borrelia* are associated with different symptoms and disease presentations. *B. garinii* is in particular neurotropic, causing neuroborreliosis while *B. afzelii* is mostly associated with ACA, and *B. burgdorferi* is most arthritogenic.

**DIAGNOSIS**

In case of a typical EM, no confirmation of infection is necessary to allow diagnosis of Lyme disease. Moreover, as antibody formation typically requires 2 to 6 weeks, most serological tests are negative the first weeks after infection. For all other manifestations of Lyme disease, two-tier serological testing is performed. Usually an enzyme linked immunosorbent assay (ELISA) is performed as a screening test. When positive, IgM and IgG immunoblotting should be performed to confirm infection. A positive serological test is not always indicative of active Lyme borreliosis, as antibodies can persist for a long time, even after successful treatment. When more diagnostic certainty is needed, for example when high background rates of seropositivity exist, additional testing can be necessary. In the case of neuroborreliosis, a lumbar puncture should be performed to assess whether borrelial antibodies are locally produced in the CNS (intrathecal antibody production). Of note, intrathecal antibodies can also persist for months to years after successful antimicrobial treatment. When Lyme arthritis is suspected, PCR testing of joint fluid or synovium can be done to detect DNA of *Borrelia*, although a negative result does not rule out Lyme borreliosis.

Isolating the microorganism is the gold standard for infectious diseases. However, culture of *Borrelia* is cumbersome, especially from other sites than the skin. Other tests, such as *Borrelia*-induced T-lymphocyte proliferation, CD57 NK cell counts, live blood microscopy, urine antigen detection tests, and PCR on urine or blood, are not recommended for diagnostic purposes as their reliability has not been proven.


TREATMENT

*Borrelia* bacteria are sensitive to many types of antibiotics, including tetracycline, most penicillins, macrolides and most 2nd and 3rd generation cephalosporins. Treatment of erythema migrans is recommended to prevent or treat possible early dissemination, although an EM can spontaneously disappear. Doxycycline, amoxicillin, phenoxymethylpenicillin, and cefuroxime axetil are highly effective and appropriate choices for treatment. Macrolides are used as second-line treatment, as they were suggested to be less effective. Doxycycline is the primary drug of choice, and two trials have shown that a treatment of merely 10 days was effective. If doxycycline is contra-indicated, i.e. in young children or pregnant women, amoxicillin is an appropriate alternative.

Doxycycline is also the preferred drug to treat early disseminated Lyme borreliosis such as arthritis, with the exception of neuroborreliosis. Here, the parenteral drug ceftriaxone is preferred, as it crosses the blood-brain barrier well and has been shown highly effective. Treatment is recommended for at least 2 weeks, but may be prolonged in individual cases to 4 weeks. In addition, intravenous ceftriaxone has been recommended for patients with Lyme carditis with an atrioventricular nodal block.

For treatment of late disseminated borreliosis, doxycycline is recommended for cases of ACA or arthritis, while ceftriaxone is advised for chronic neuroborreliosis. The duration of treatment for late disseminated infection is at least 4 weeks. In patients with persistent or recurrent arthritis after a recommended course of oral antibiotic therapy, re-treatment with another 4-week course of oral antibiotics, and if not successful, a 2 to 4-week course of ceftriaxone is recommended. As Lyme arthritis may develop into a recurrent autoimmune arthritis, later stages may require non-steroidal anti-inflammatory drugs, intra-articular injection of corticosteroids, or disease-modifying anti-rheumatic drugs such as hydroxychloroquine or methotrexate.

Within 24 hours after the initiation of treatment with antibiotics for Lyme disease, about 15% of patients may have a reaction similar to the Jarisch–Herxheimer reaction (increased temperature, myalgia, and arthralgia), supposedly as a result of an increase in circulating toxins associated with lysis of spirochetes. The reaction resolves without serious consequences within 24 to 48 hours.
PERSISTENT SYMPTOMS ATTRIBUTED TO LYME DISEASE

Although most symptoms that accompany documented Lyme disease tend to disappear quickly after treatment, symptoms can persist. These borreliosis-attributed persistent symptoms are also referred to as post-Lyme disease syndrome (PLDS), or – among patients – as chronic Lyme disease. In 2006, the Infectious Diseases Society of America (IDSA) has proposed a definition for PLDS. This definition was supported by their European partner, the European Union Concerted Action on Lyme Borreliosis (EUCALB) in 2011. Persistent symptoms include fatigue, widespread musculoskeletal pain (arthralgia, myalgia), and cognitive complaints such as disturbances in concentration and memory. They exist for at least 6 months, and began within 6 months after documented Lyme disease (e.g. EM, neuroborreliosis, arthritis). Furthermore, the documented episode of Lyme disease should have been treated as recommended by the guidelines.

In the Netherlands, general practitioners have reported more than 900 cases of patients with persistent borreliosis-attributed symptoms in 2010 (5.5 per 100,000 inhabitants). Of all Lyme patients included in 13 randomized treatment trials performed in the USA and Europe, approximately 10 to 15% have reported persistent symptoms. A meta-analysis of 5 studies showed that the prevalence of these persistent symptoms was higher in patients with a history of Lyme disease compared to controls. However, a prospective study comparing patients with a treated EM to a healthy control group, showed similar percentages after 6-12 months. Symptoms in the patient group were more severe than in the control group.

There are many hypotheses concerning the pathophysiology of persistent symptoms. An ongoing infection with Borrelia burgdorferi or other pathogens (co-infections) after antibiotic treatment has been suggested as well as a persistent abnormal immune response. Furthermore, it has been suggested that beliefs and behavior of patients with persistent borreliosis-attributed symptoms might be related to functional outcome.

Due to the diverse hypotheses, the treatment of persistent borreliosis-attributed symptoms has been subject of an ongoing debate since the end of the past century. Some advocate prolonged antibiotic treatment, as they consider persistent symptoms to be an expression of persistent infection. However, many others see these complaints in the light of a post-infectious syndrome or an infection-induced auto-immune reaction and recommend no
additional anti-microbial therapy. One of the reasons for this unrelenting conflict of opinions is that there is no hard endpoint to assess microbiological cure. Anti-

*Borrelia* IgG typically remains present up to years after infection, or even lifelong. Positive serology merely points out that patients have been once infected with *Borrelia*. Consequently, we are mostly left with measurement of quality of life for the assessment and follow-up of persistent borreliosis-attributed symptoms.

**TREATMENT OF PERSISTENT SYMPTOMS ATTRIBUTED TO LYME DISEASE**

Several studies have investigated the effect of prolonged antibiotic therapies for persistent borreliosis-attributed symptoms. Three open cohort studies have been published, describing antimicrobial therapy for 1 to 11 months. Donta et al. reported that prolonged therapy with oral doxycycline for a median of 4 months was associated with success in a large case series of 277 patients. Success was defined as more than 75% improvement of functioning, and cure if a patient reported no symptoms more than a year after stopping treatment. Of all patients, 20% were fully cured, and another 70% improved. In another case series by the same author, the combination of clarithromycin and hydroxychloroquine was used, as hydroxychloroquine is hypothesized to increase macrolide activity by increasing the lysosomal pH. This case series of 235 patients reported that combined therapy for at least 3 months (median 4 months) may be at least as effective as prolonged doxycycline, as 77% improved. Most patients already improved within 2 weeks after initiation of therapy, and all patients had improved after 3 months (Table 1). Of note, in both studies, inclusion criteria were not clearly defined, and disease definition was not according to common standards. To be eligible, patients had to have symptoms for at least 3 months, and serologic reactivity against *Borrelia burgdorferi*, but no documented Lyme disease was required.
TABLE 1. Prior symptom duration vs onset of improvement in the treatment of chronic Lyme disease.
(adapted from Donta et al. 2003, Table 5)

<table>
<thead>
<tr>
<th>Time to first improvement (in weeks)</th>
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<tbody>
<tr>
<td>Prior symptom duration</td>
</tr>
<tr>
<td>&lt;1 yr (n=25)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>1-3 yr (n=39)</td>
</tr>
<tr>
<td>&gt;3 yr (n=92)</td>
</tr>
<tr>
<td>All durations (n=156)</td>
</tr>
<tr>
<td>Cumulative percentage of improved patients</td>
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Clarissou et al. prospectively followed 100 patients who were treated for 3 to 6 months with amoxicillin (39%), ceftriaxone (31%), or doxycycline (27%)\(^{54}\). Overall, the number and intensity of symptoms decreased in time. However, disease definition was not standardized, and less relevant symptoms such as gastro-intestinal and respiratory complaints were also included as endpoints.

Although these publications were suggestive of an effect of antibiotic treatment, no conclusions can be drawn from these three studies, as they were uncontrolled, observational studies, and none used standardized questionnaire outcomes. More reliable information can be obtained from prospective, controlled studies that compare different treatments. Five randomized clinical trials have been performed on persistent borreliosis-attributed symptoms, resulting in 6 publications\(^ {58-63}\). Unless otherwise stated, study subjects were patients with persistent borreliosis-attributed symptoms for at least 3 to 6 months, including arthralgia, musculoskeletal pain, cognitive complaints and/or sensory disturbances, that arose after documented Lyme disease with positive serology, for which patients had received the recommended treatment.

The only study that was performed in Europe compared 3 weeks of ceftriaxone followed by placebo to 3 weeks of ceftriaxone followed by oral amoxicillin for 100 days in 145 patients, with a follow-up of 12 months\(^ {63}\). No difference between groups was shown, but its results have to be interpreted with care as the inclusion criteria were poorly defined, as
was the primary endpoint, which was a self-composed VAS scale for symptom severity. In addition, the study was underpowered as it was stopped prematurely due to slow inclusion.

The other four randomized placebo-controlled blinded trials are considered most valuable. They investigated whether antibiotic treatment was more effective than placebo. The length of treatment differed from 4 weeks to 10 weeks. Most studies used ceftriaxone, but Cameron et al. compared amoxicillin to placebo and Klempner et al. compared placebo to one month of ceftriaxone followed by 2 months of doxycycline. Outcome measures varied as well.

The trials by Klempner et al. could not demonstrate any beneficial effects of prolonged antibiotic treatment on quality of life in 115 patients with post-treatment Lyme disease, either seronegative or seropositive for *Borrelia* IgG antibodies. The primary outcome measures were the physical- and mental-health-component summary scales of the Medical Outcomes Study 36-Item Short-Form General Health Survey (SF-36). These scales measured the health-related quality of life, on day 180 of the study. The SF-36 scores were not significantly different in the group randomized to 2 weeks of ceftriaxone followed by 2 months doxycycline, compared to the matching placebo group. However, the results of this study have been subject of debate, as the number of patients included was small, and it has been suggested to be underpowered as a result of an optimistic estimate of treatment effect size.

Fallon et al. randomized 37 Lyme patients with positive *Borrelia* IgG Western blot and memory impairment despite three weeks of ceftriaxone to an additional 10 weeks of ceftriaxone (23 patients) versus placebo (14 patients). At 12 weeks, they reported a significantly greater effect on objective neurocognitive functioning in the ceftriaxone group. Although this effect did not sustain for 3 months, the effect on the secondary outcomes physical functioning and pain did sustain among the severely impaired patients. However, the question is how representative this study is for the real-life situation, as participants were highly selected and needed to have objectified memory loss.

Kaplan et al. have investigated objective neurocognitive function as well as subjective functioning as a secondary outcome of the trial by Klempner et al. Although there was improvement in subjective cognitive functioning, measured with a scale derived from the Medical Outcome Study (MOS), no improvement on objective functioning was seen in this study with 98 patients.
Krupp et al. assessed cognitive function and fatigue in a double-blind randomized trial of 4 weeks of ceftriaxone versus placebo in a small group of 55 patients with persisting fatigue after at least three weeks of antibiotics. At the primary endpoint after 6 months, no improvement in neuropsychological performance (focus on mental speed) was seen. However, a positive effect of ceftriaxone was found on fatigue at the primary endpoint, measured with the Fatigue Severity Scale FSS-11.

Cameron et al. investigated the effect of oral amoxicillin for 3 months compared to placebo in a double-blind randomized trial in 84 patients with recurrence of persistent symptoms (arthralgia, arthritis, cardiac or neurologic involvement) after previous treatment. They measured quality of life using the SF-36 questionnaire. The study was terminated prematurely, and the publication did not report on the primary endpoint of SF-36 results in the intention-to-treat population. In the subgroup of 45 patients with successful outcomes, those randomized to amoxicillin reported an improvement in mental quality of life that was significantly larger than in the placebo group. However, there was no difference in physical quality of life between the groups. The results need to be interpreted with caution as the study had a high drop-out rate, and outcomes were only reported for the subgroup of successfully treated patients.

In summary, there are few prospective, controlled data to support prolonged antibiotic treatment in patients with persistent symptoms attributed to Lyme disease. Some studies have suggested positive outcomes on selected endpoints. Other randomized clinical trials did not demonstrate beneficial effects of prolonged antibiotic treatment. However, most studies have received considerable methodological criticism, and the results were generally disappointing and not generalizable.

OUTLINE OF THE THESIS

The aim of this thesis is to gain more insight into persistent symptoms that are attributed to Lyme disease and the effect of antibiotic treatment on quality of life as well as cognitive performance and whether longer-term treatment is cost-effective.
In chapter 1, we depicted a framework and provided background for this thesis.

In chapter 2, we elaborate on the design of our clinical trial, the Persistent Lyme Empiric Antibiotic Study Europe (PLEASE). Our randomized placebo controlled clinical trial was performed to assess the effect of long-term antibiotic treatment on persistent symptoms attributed to Lyme borreliosis. As previous trials were associated with methodological problems, and as their results were inconsistent, we designed a European trial that did not have the shortcomings of previous studies.

In chapter 3, we assess the effect of longer-term antibiotic treatment on the quality of life of persistent symptoms attributed to Lyme disease, the main outcome of the PLEASE trial.

Chapter 4 describes the cost-effectiveness of the intervention.

In chapter 5, we describe cognitive symptoms and cognitive performance of patients with persistent symptoms that are attributed to Lyme disease, compared to the general population.

The effect of antibiotic treatment on cognitive performance will be described in chapter 6.

Finally, in chapter 7 we elaborate on the role of expectancies and other individual difference factors in predicting quality of life course after antimicrobial therapy for persistent symptoms attributed to Lyme disease.

We conclude this thesis with a summary and general discussion in chapter 8.
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Chapter 1


Introduction and outline of the thesis


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Persistent Lyme Empiric Antibiotic Study Europe (PLEASE) - design of a randomized controlled trial of prolonged antibiotic treatment in patients with persistent symptoms attributed to Lyme borreliosis

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ABSTRACT

Background: Lyme borreliosis, a potentially severe tick-borne infection caused by Borrelia burgdorferi, can cause multi-system inflammatory disease. The incidence has been increasing, as has the number of patients with persistent symptoms attributed to Borrelia. These symptoms, also referred to as post-Lyme disease syndrome, may follow an erythema migrans or other Lyme manifestations, and include pain, fatigue, and cognitive disturbances. The optimal duration of treatment for these symptoms is a subject of controversy. The PLEASE study is designed to determine whether prolonged antibiotic treatment leads to better patient outcome than standard treatment.

Methods/design: The PLEASE study is a double-blind, randomized, placebo-controlled trial. Based on power analysis and compensating for possible loss to follow-up, a minimum of 255 patients with borreliosis-attributed persistent symptoms are included. These symptoms are either (a) temporally related to an erythema migrans or otherwise proven symptomatic borreliosis, or (b) accompanied by a positive B. burgdorferi IgG or IgM immunoblot. All patients receive open-label ceftriaxone for two weeks. Patients are then randomized (ratio 1:1:1) to blinded oral follow-up treatment for 12 weeks with (I) doxycycline, (II) clarithromycin combined with hydroxychloroquine, or (III) placebo. The primary outcome is the physical component summary score (PCS) of the RAND-36 Health Status Inventory (RAND SF-36) at week 14. Secondary outcomes include physical and mental aspects of health-related quality of life (assessed by the subscales of the RAND SF-36), fatigue, neuropsychological evaluation, physical activity, and cost-effectiveness.

Discussion: This article describes the background and design issues of the PLEASE study protocol. The results of this study may provide evidence for prescribing or withholding prolonged antibiotic treatment.

Trial registration: ClinicalTrials.gov: NCT01207739, Netherlands Trial Register: NTR2469
BACKGROUND

Lyme borreliosis, the most common tick-borne infection in America, Europe, and Northern Asia, is a multi-system inflammatory disease caused by the spirochete *Borrelia burgdorferi sensu lato*. During the past two decades, the incidence has been increasing. In the USA, the number of reported borreliosis cases has doubled from 9,908 cases in 1992 to 19,931 in 2006. Incidence rates vary per state but have increased substantially over the last decade, with an incidence up to 75 cases per 100,000 persons in 2012. In the Netherlands, the incidence of the most common form of borreliosis, erythema migrans (EM), has risen from 39 per 100,000 in 1994 to 134 per 100,000 persons in 2009.

In parallel with the growing incidence of early Lyme disease, the number of patients with persistent symptoms attributed to infection with *B. burgdorferi* seems to increase as well. These borreliosis-attributed persistent symptoms, also referred to as post-Lyme disease syndrome, chronic Lyme disease, or (true or presumed) persistent Lyme disease, may follow an EM or other, possibly unnoticed, manifestations of early Lyme disease, regardless of initial appropriate antibiotic treatment. Patients mainly present with pain, fatigue, neurological, and cognitive disturbances. Three months after treatment of an EM, the prevalence of these symptoms can be as high as 25%. Although this percentage tends to decrease as more time elapses, symptoms are often disabling, and influence the daily life of these patients. Especially chronic pain has been shown to be an important contributor to impairment of health-related quality of life, and is similar to that reported by patients with osteoarthritis.

So far, no general, well-accepted definition of the syndrome of borreliosis-associated persistent symptoms exists. This has resulted in a lack of data on its incidence and prevalence, and has contributed to confusion and controversy. This controversy especially relates to the pathogenesis of borreliosis-attributed persistent symptoms: whether they emerge from an ongoing infection, are a post-infectious problem, or are not related to a *Borrelia* infection at all. Currently available diagnostic tools (primarily based on serology) are appropriate for the diagnosis of early Lyme disease in most cases, but have little value for the diagnosis of potentially persistent *Borrelia* infection. As IgG antibodies against *Borrelia* may persist for many months or even years after acute infection, positive serology is not an indicator of active or persistent *Borrelia* infection. As long as there is no specific laboratory test for active infection, the decision whether and how long patients with persistent
Symptoms should be treated depends on evidence from clinical studies. However, as this evidence has not been consistent, two different approaches exist for patients with borreliosis-attributed persistent symptoms: (1) standard short-term treatment for 2–4 weeks, as advised for most manifestations of Lyme borreliosis by the Infectious Diseases Society of America (IDSA) or (2) long-term treatment for at least 3 months, as advised by the International Lyme and Associated Diseases Society (ILADS). Previous randomized clinical trials have not convincingly demonstrated beneficial effects of prolonged antibiotic treatment, and have been subject of ongoing debate.

To obtain more insight into the optimal treatment regimen for patients with borreliosis-attributed persistent symptoms, we designed a double-blind, randomized clinical trial to compare short- versus long-term treatment. In this 3-arm study, entitled Persistent Lyme Empiric Antibiotic Study Europe (PLEASE), ceftriaxone followed by doxycycline (arm 1) or ceftriaxone followed by the combination of clarithromycin and hydroxychloroquine (arm 2) are compared to short-term therapy with ceftriaxone followed by placebo (arm 3). Here, we describe the study protocol.

**METHODS / DESIGN**

**STUDY DESIGN**

A randomized, double-blind, placebo-controlled trial is performed to determine whether long-term antibiotic treatment (ceftriaxone followed by doxycycline or ceftriaxone followed by the combination of clarithromycin and hydroxychloroquine) leads to better patient outcome than short-term treatment (ceftriaxone followed by placebo) in patients with borreliosis-attributed persistent symptoms. This prospective 3-arm study is conducted at two sites in the Netherlands, the Radboud university medical center (Radboudumc) and the Sint Maartenskliniek, and has been approved by the Medical Ethics Review Committee CMO Regio Arnhem-Nijmegen (registration number 2009/187, NL27344.091.09). The study is conducted in accordance with the principles stated in the most recent version of the Declaration of Helsinki and the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice.
STUDY POPULATION

All patients are recruited from the outpatient clinic of the Radboudumc, after nationwide referral by physicians. The Radboudumc serves as one of the tertiary referral centers for the Netherlands’ population of around 17 million. Screening is done using standard clinical and laboratory protocols. Eligibility is assessed by a physician according to specific inclusion and exclusion criteria (Table 1). In short, patients with borreliosis-attributed persistent symptoms (musculoskeletal pain, arthritis, arthralgia, neuralgia, sensory disturbances, or neuropsychological/cognitive disorders, with or without persistent fatigue) are eligible if these symptoms are either (a) temporally related to an erythema migrans or otherwise proven symptomatic borreliosis, or (b) accompanied by a positive *B. burgdorferi* IgG or IgM immunoblot. An eligible patient is asked to sign informed consent after obtaining written information about the study.

RANDOMIZATION AND BLINDING

After obtaining informed consent and completing the baseline assessment, patients are randomly assigned to one of three groups in a 1:1:1 allocation ratio (Figure 1). The randomization is computerized and balanced by minimization for age (<or ≥40 years), gender, duration of symptoms (<or ≥1 year), and baseline Global Health Composite score of the RAND-36 Health Status Inventory (RAND SF-36), consisting of all RAND SF-36 subscales. The randomization list consists of consecutive medication numbers that are entered into a secured web-based database by an independent web manager. All personnel involved in the study (except the web manager and study pharmacist) and participants are masked to treatment allocation. If the code is broken, it renders the patient non-eligible. To assess success of masking, patients are asked at the week 14 evaluation whether they think they have received oral antibiotics or placebo.
Table 1. Inclusion and exclusion criteria

**Inclusion criteria**

<table>
<thead>
<tr>
<th>1</th>
<th>Males or non-pregnant, non-lactating females who are 18 years or older</th>
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<tr>
<td>2</td>
<td>Complaints of musculoskeletal pain, arthritis, arthralgia, neuralgia, sensory disturbances (such as paraesthesias or dysesthesias), or neuropsychological/cognitive disorders, with or without persistent fatigue, that are:</td>
</tr>
<tr>
<td>A</td>
<td>either temporally related to an episode of erythema migrans or otherwise proven symptomatic Lyme borreliosis (defined as within 4 months after erythema migrans as assessed by a physician, or positive biopsy, PCR, culture, or intrathecal B. burgdorferi antibodies)</td>
</tr>
<tr>
<td>B</td>
<td>or accompanied by a positive B. burgdorferi IgG or IgM immunoblot (as defined by strict criteria in line with the European Union Concerted Action on Lyme Borreliosis (EUCALB) and the manufacturer of the immunoblot*, 20,21), regardless of prior ELISA IgG/IgM screening results</td>
</tr>
<tr>
<td>3</td>
<td>Subjects must sign a written informed consent form</td>
</tr>
</tbody>
</table>

**Exclusion criteria**

| 1 | Subjects with a known history of allergy or intolerance to tetracyclines, macrolides, hydroxychloroquine, or ceftriaxone |
| 2 | Subjects who have had more than 5 days of antimicrobial therapy with activity against B. burgdorferi within the previous 4 weeks |
| 3 | Subjects with a presumed diagnosis of neuroborreliosis (CSF pleiocytosis or intrathecal antibody production) for which intravenous antimicrobial therapy is required |
| 4 | Subjects with a known diagnosis of HIV-seropositivity or other immune disorders |
| 5 | Subjects with positive syphilis serology or signs of other spirochetal diseases |
| 6 | Subjects with moderate or severe liver disease defined as ALP, ALT, or AST greater than 3 times upper limit of normal |
| 7 | Subjects who are receiving and cannot discontinue cisapride, astemizole, terfenadine, barbiturates, phenytoin, or carbamazepine |
| 8 | Subjects who are currently enrolled on other investigational drug trials or receiving investigational agents |
| 9 | Subjects who have been previously randomized into this study |
| 10 | Severe physical or psychiatric co-morbidity that interferes with participation in the study protocol, including previous medical diagnosis of rheumatic conditions, chronic fatigue syndrome, or chronic pain conditions, as well as insufficient command of the Dutch language |
| 11 | Co-morbidity that could (partially) account for the symptoms of the subject (e.g., vitamin B12 deficiency, anemia, hypothyroidism) |
| 12 | Subjects of child-bearing potential unwilling to use contraception methods other than oral contraceptives during the study therapy period |

**Abbreviations:** PCR = polymerase chain reaction, CSF = cerebrospinal fluid, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase  
* EUROLINE-WB: Anti-Borrelia (whole antigen plus recombinant VlsE). EUROIMMUN Corporation, Lübeck, Germany.
INTERVENTION

All patients are treated with open-label intravenous (i.v.) ceftriaxone 2000 mg qd via a peripheral i.v. catheter for 14 days. To monitor side effects, patients are admitted to the Sint Maartenskliniek for administration during day 1 and 2. Subsequent doses, prepared by the Sint Maartenskliniek Pharmacy, are given intravenously in a home-care setting by specialized nurses. After completion of ceftriaxone treatment, patients start with the randomized, blinded, oral study drugs. The oral drug regimen comprises either (I) doxycycline 100 mg b.i.d. combined with a placebo b.i.d. for 12 weeks, (II) clarithromycin 500 mg b.i.d. combined with hydroxychloroquine 200 mg b.i.d. for 12 weeks, or (III) double placebo b.i.d. for 12 weeks. The study drugs are to be taken twice daily after the meals. Study drugs and placebo are prepared as capsules with identical appearance. Preparation and labeling of doxycycline, clarithromycin, hydroxychloroquine, and placebo is performed by the Clinical Trials Unit of the Department of Clinical Pharmacy of the Radboudumc according to Good Manufacturing Practice (GMP) guidelines. Drug utilization is assessed by pill counting. Compliance is verified by using patient diaries and MEMS (Medication Event Monitoring System) caps 23,24.

CONCOMITANT MEDICATION

Any antibacterial drugs other than study medications are prohibited during the entire study period. In case of proven intercurrent infections (e.g., urinary tract infection), specific antimicrobial therapy may be given for a maximum of 5 days. Indications should be discussed with the investigator, and efforts should be made to select an antimicrobial drug with no in vitro activity against B. burgdorferi. The following drugs are prohibited because of potential interaction with study drugs or potential effects on efficacy of treatment: cisapride, astemizole, terfenadine, barbiturates, phenytoin, carbamazepine, prednisone, recombinant cytokines, hematopoietic growth factors, or immunoglobulins. If treatment with one of these drugs is required, the patient will be classified as therapy discontinuation.
FIGURE 1. Flowchart trial design.
ASSESSMENTS

An extensive baseline assessment is performed including questionnaires, measurement of physical activity, and clinical, laboratory, microbiological, and neuropsychological evaluation. Neurological symptoms are assessed by the lead study physicians using a standardized interview and clinical neurological examination at baseline and week 14.

Study visits for safety evaluation are performed at week 2, week 8, and week 14 after baseline. Safety assessments include a medical history, physical examination, and laboratory investigation (hemoglobin, hematocrit, leukocytes, platelets, glucose, creatinin, alkaline phosphatase, alanine aminotransferase).

Efficacy evaluation is performed at week 14 (end of treatment period, EOT), week 26 (12 weeks after EOT), and week 40 after baseline (end of study, EOS, 26 weeks after EOT). After the last comprehensive outcome assessment at week 40, patients are surveyed by post-study questionnaires at week 52.

OUTCOME MEASURES

The primary outcome measure is health-related quality of life at EOT (week 14), assessed by the physical component summary score (PCS) of the RAND-36 Health Status Inventory (RAND SF-36). This score is based on the weighed subscale scores of the four physical RAND SF-36 subscales (physical functioning, role limitations due to physical health problems, pain, and general health perceptions). The PCS is transformed to norm-based T-scores (with a mean of 50 and a standard deviation of 10 in the general population) and ranges from 15 to 61, with higher scores indicating a better physical quality of life.

Main secondary endpoints include:

(a) Physical and mental aspects of health-related quality of life, assessed by the subscales of the RAND SF-36 (physical functioning, role limitations due to physical health problems, pain, general health perceptions, emotional well-being (also known as mental health), role limitations due to emotional problems, social functioning, and energy/fatigue (also known as vitality).

(b) Fatigue, assessed by the Fatigue Severity subscale of the Checklist Individual Strength (CIS). The CIS is a reliable instrument with good validity and sensitivity to change in patients with rheumatoid arthritis, fibromyalgia, and chronic fatigue syndrome.
(c) Neuropsychological assessment covering the five major cognitive domains, based on a similar test battery previously used to measure borreliosis-related impairment. 

*Episodic memory* is assessed using the Rey Auditory Verbal Learning Test, using parallel versions for the follow-up assessments to reduce nonspecific learning effects. *Attention/Working memory* is assessed using the Digit Span test. *Language* is measured with the Category Fluency test (animal/profession naming). *Speed of information processing* is assessed using the Trail Making Test (TMT) part A, the average speed of Cards I and II from the Stroop Color-Word Test, and the Symbol-Digit Substitution Test. *Executive functions* are measured using the TMT Interference score (Part B/Part A) and the Stroop interference score (Card III/average of Cards I and II). To identify participants who display suboptimal effort affecting symptom validity, the Amsterdam Short Term Memory Test is administered at baseline. The entire test battery requires approximately 1 hour to be completed and is performed according to a standardized protocol by three psychologists, who have been trained in test administration and scoring.

(d) Physical activity during 12 days, measured by an actometer. An actometer is a three-dimensional motion device with a piezoelectric sensor that is worn around the ankle. Sensor signals are stored every five minutes, from which mean Daily Physical Activity scores are computed. Actometers have been shown to yield valid and highly reliable data.

**ECONOMIC EVALUATION**

To determine the cost-effectiveness of the different antibiotic regimens, an economic evaluation is conducted, and these results will be published separately. This cost-utility analysis investigates the potential efficiency of short-term antibiotic therapy (2 weeks) versus long-term antibiotic therapy (14 weeks) from a societal perspective. Primary outcome measures are costs and quality-adjusted life years (QALYs). For the overall quantification of health status as a single index, the Dutch version of the standard EQ-5D classification system developed by the EuroQol Group is used. QALYs will be estimated from the EQ-5D scores over a one-year period using the trapezium method.

The cost analysis consists of two main parts. First, volumes of care are measured prospectively using a structured survey. Productivity losses for patients are estimated using the Short Form - Health and Labour Questionnaire (SF-HLQ). The friction cost method
will be applied. In the second part of the cost analysis, prices will be determined for each unit of care consumed using the Dutch manual for cost research. The cost-effectiveness analysis will consist of computing the incremental cost effectiveness ratio (ICER) by dividing the mean difference in total costs by the mean difference in QALYs. Insight into parameter uncertainty will be obtained with the bootstrap method and will be presented as cost-effectiveness acceptability curves.

SAFETY MONITORING
Safety is evaluated by clinical laboratory tests and physical examinations. All observed and reported adverse events, regardless of suspected causal relationship, are recorded. An independent external data and safety monitoring board (DSMB) will review the blinded safety data after the first 60 patients have reached the end of treatment study visit. The DSMB may decide to recommend study termination or protocol modifications if required by the safety data or trial conduct.

STATISTICAL ANALYSIS
Data will be analyzed according to the modified intention-to-treat (mITT) principle. Patients who have been randomized into the study and received at least one dose of ceftriaxone are included in the mITT analysis group. In the primary analysis, analysis of covariance (ANCOVA) will be used to compare the three study arms, with gender and the baseline value of the dependent variable as covariates. Pairwise comparisons are performed for the different treatment modalities with Bonferroni correction for multiple comparisons. Missing data will be imputed by carrying the last observation forward, in order to obtain a conservative estimate of the treatment effect. No interim efficacy analysis will be performed. Two-sided 5% significance levels will be used to identify statistically significant results. All confidence intervals reported will be 95% confidence intervals. All statistical analyses will be performed using SPSS software.

A sensitivity analysis will be performed on the per-protocol subgroup. The per-protocol population comprises of patients for whom all of the following apply: has met the in- and exclusion criteria; has taken at least 75% of the study drugs as recorded by MEMS; has not taken any other antimicrobial drug for more than 5 days during the study period; has not taken any prohibited concomitant medication; has not been unblinded before end of study.
When a difference between one of the experimental treatments (ceftriaxone followed by 12 weeks of either of the oral treatments) and the reference treatment (two weeks ceftriaxone followed by placebo) is found, subgroup analyses will be performed to identify factors that may affect the treatment outcome. This will be done by adding the factors and their interaction with the treatment to the analysis of covariance model. The duration of treatment effect will be evaluated in an explorative way with linear mixed models. When an outcome variable is measured more than once, a random (patient-dependent) intercept will be included in the analysis.

To evaluate the neuropsychological outcomes, results on individual tests will be standardized into z-scores to make across-test comparison possible (using baseline group mean and standard deviation as reference), and averaged into cognitive domain scores. Higher z-scores reflect a better performance. If necessary, scales will be inverted, e.g., in the case of reaction times where higher scores reflect a slower performance.

**SAMPLE SIZE**

The final power calculation was based on a pilot study on 80 patients with borreliosis-attributed persistent symptoms (Berende et al., unpublished). Patients were classified as having a poor or reasonable clinical condition as assessed during the first clinical consultation at the outpatient clinic. The difference in the PCS score between patients with a poor and those with a reasonable clinical condition was 3 points, with a standard deviation of 8. This corresponds with the minimally clinically important difference (MCID) of 2 to 5 points that has been proposed for the PCS. In order to detect a difference of 3 points with a power of 90%, a two-sided alpha of 5% and a reliability coefficient (correlation between consecutive measurements) of 0.7, a minimum of 75 patients are required per treatment group (225 patients in total). To compensate for possible loss to follow-up, a study population of at least 255 patients is targeted for.
The PLEASE study evaluates whether long-term antibiotic treatment of patients diagnosed with borreliosis-attributed persistent symptoms is efficient and leads to better patient outcome than short-term treatment. So far, there are few prospective, controlled data to support prolonged antibiotic treatment. Indeed, some studies have suggested positive outcomes on selected endpoints, such as persistent fatigue, cognitive functioning, quality of life, or clinical response rate, in specific groups of patients with putative persistent Lyme disease. However, these results were generally disappointing, and cannot be generalized. Other randomized clinical trials have not demonstrated beneficial effects of prolonged antibiotic treatment. Importantly, all of these studies were performed in North America. Borreliosis is caused by different Borrelia species in the US and Europe, with different clinical manifestations. The present study will be the first randomized clinical trial to study long-term antibiotic treatment for borreliosis-attributed persistent symptoms in Europe.

The strategic choices leading to the design of a prospective, randomized, 3-arm study are complex. First, i.v. ceftriaxone followed by doxycycline is generally considered the gold standard therapy for complicated borreliosis. Whereas administration of ceftriaxone for longer than 2 weeks has been advocated, a randomized, open-label study was unable to demonstrate that ceftriaxone treatment for 4 weeks would be significantly better.

Prolonged therapy with oral doxycycline has been associated with success in a large case series of patients with borreliosis-attributed persistent symptoms. Data from another case series suggested that combined therapy with oral clarithromycin and hydroxychloroquine for at least 3 months may be at least as effective as prolonged doxycycline. Hydroxychloroquine increases the lysosomal pH and is hypothesized to increase macrolide activity. However, few conclusions can be drawn from those clinical studies, as they were retrospective, uncontrolled, observational studies. Based on these considerations, the present study was designed to compare a 12 weeks’ course of doxycycline to 12 weeks of clarithromycin and hydroxychloroquine versus placebo.

To provide a standard treatment for all patients, and to cover potentially undiagnosed neuroborreliosis, all randomized patients receive an open-label course of i.v. ceftriaxone for 2 weeks preceding randomized blinded study drugs. In this respect, the present study
differs from previous trials comparing prolonged therapy to placebo \cite{10,17,18,29}. By applying a standardized open-label treatment to patients in all treatment arms, the study is designed to compare short-term standard treatment \cite{15} to prolonged therapy as advocated by several position papers \cite{16,53}. In addition, this approach does not leave potentially active infection untreated in patients who are randomized to the control arm, and it also controls for the wide variation in prior antibiotic therapies (or lack thereof) that patients with borreliosis-attributed persistent symptoms may have received.

As the primary outcome measure, we have chosen the physical component summary score (PCS) of the RAND-36 Health Status Inventory (RAND SF-36) \cite{22}. The RAND SF-36 is similar to the Medical Outcomes Study (MOS) 36-item Short-Form General Health Survey (SF-36) \cite{54}. The PCS, also known as the physical health composite score (PHC) \cite{22}, is computed by a non-orthogonal scoring algorithm. Several previous studies have used the alternate (SF-36) version of the PCS, applying a principal components analysis with orthogonal factors, with mental health components contributing negatively to this PCS score \cite{54}. This SF-36 PCS has proven difficult to interpret as the level of mental health influences the physical health score and is therefore not purely a reflection of physical health. Furthermore, the SF-36 PCS is less sensitive to change than the underlying scales, while the RAND SF-36 PCS has been shown to be sensitive to change \cite{55-62}. Despite the differences in calculation of both composite scores, they do correlate highly, indicating that they do represent similar constructs \cite{58,59}.

In conclusion, the PLEASE study is expected to provide evidence for prescribing or withholding prolonged antibiotic treatment as compared to standard short-term treatment in patients with borreliosis-attributed persistent symptoms. In addition, this study may help to define subgroups of patients who may or may not benefit from additional antibiotic treatment, and contribute to a more cost-effective management of this disease entity.

**ACKNOWLEDGEMENTS**

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2. CDC Lyme Disease Statistics. [http://www.cdc.gov/lyme/stats/].


Randomized trial of longer-term therapy for symptoms attributed to Lyme disease

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Fidel J. Vos
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Michiel L. Vogelaar
Mirjam Tromp
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ABSTRACT

Background: The treatment of persistent symptoms attributed to Lyme disease remains controversial. We assessed whether longer-term antibiotic treatment of persistent symptoms attributed to Lyme disease leads to better outcomes than does shorter-term treatment.

Methods: In a randomized, double-blind, placebo-controlled trial conducted in Europe, we assigned patients with persistent symptoms attributed to Lyme disease - either related temporally to proven Lyme disease or accompanied by a positive IgG or IgM immunoblot assay for Borrelia burgdorferi - to receive a 12-week oral course of doxycycline, clarithromycin plus hydroxychloroquine, or placebo. All study groups received open label intravenous ceftriaxone for 2 weeks before initiating the randomized regimen. The primary outcome measure was health-related quality of life, as assessed by the physical-component summary score of the RAND-36 Health Status Inventory (RAND SF-36) (range, 15 to 61, with higher scores indicating better quality of life), at the end of the treatment period at week 14, after the 2-week course of ceftriaxone and the 12-week course of the randomized study drug or placebo had been completed.

Results: Of the 281 patients who underwent randomization, 280 were included in the modified intention-to-treat analysis (86 patients in the doxycycline group, 96 in the clarithromycin–hydroxychloroquine group, and 98 in the placebo group). The SF-36 physical-component summary score did not differ significantly among the three study groups at the end of the treatment period, with mean scores of 35.0 (95% confidence interval [CI], 33.5 to 36.5) in the doxycycline group, 35.6 (95% CI, 34.2 to 37.1) in the clarithromycin–hydroxychloroquine group, and 34.8 (95% CI, 33.4 to 36.2) in the placebo group (P = 0.69; a difference of 0.2 [95% CI, -2.4 to 2.8] in the doxycycline group vs. the placebo group and a difference of 0.9 [95% CI, -1.6 to 3.3] in the clarithromycin–hydroxychloroquine group vs. the placebo group); the score also did not differ significantly among the groups at subsequent study visits (P = 0.35). In all study groups, the SF-36 physical-component summary score increased significantly from baseline to the end of the treatment period (P<0.001). The rates of adverse events were similar among the study groups. Four serious
adverse events thought to be related to drug use occurred during the 2-week open-label ceftriaxone phase, and no serious drug-related adverse event occurred during the 12-week randomized phase.

**Conclusions:** In patients with persistent symptoms attributed to Lyme disease, longer-term antibiotic treatment did not have additional beneficial effects on health-related quality of life beyond those with shorter-term treatment. (Funded by the Netherlands Organization for Health Research and Development ZonMw; PLEASE ClinicalTrials.gov number, NCT01207739.) Longer-term antibiotic treatment has no additional beneficial effects on health-related quality of life compared to shorter-term treatment in patients with persistent symptoms attributed to Lyme disease. (Funded by the Netherlands Organization for Health Research and Development; PLEASE ClinicalTrials.gov number, NCT01207739).
INTRODUCTION

Patients with Lyme disease, which is caused by the *Borrelia burgdorferi* sensu lato complex (including *B. afzelii* and *B. garinii* in Europe), often report persistent symptoms. These symptoms are also referred to as the post-Lyme disease syndrome or chronic Lyme disease and may occur after resolution of an erythema migrans rash or after other - possibly unnoticed - manifestations of early Lyme disease, regardless of whether a patient received initial appropriate antibiotic treatment. Patients present mainly with pain, fatigue, and neurological or cognitive disturbances.

Previous randomized, clinical trials have not shown convincingly that prolonged antibiotic treatment has beneficial effects in patients with persistent symptoms attributed to Lyme disease. Nonetheless, the debate about this issue has continued. Although most guidelines do not recommend antimicrobial therapy for longer than 2 to 4 weeks, other guidelines recommend prolonged antibiotic therapy.

We performed a double-blind, randomized clinical trial (Persistent Lyme Empiric Antibiotic Study Europe [PLEASE]) that included three study groups to compare shorter-term treatment (ceftriaxone followed by placebo [placebo group]) with longer-term treatment (ceftriaxone followed by doxycycline [doxycycline group] or ceftriaxone followed by the combination of clarithromycin and hydroxychloroquine [clarithromycin–hydroxychloroquine group]).

METHODS

STUDY OVERSIGHT

The trial was approved by the medical ethics review committee Commissie Mensgebonden Onderzoek regio Arnhem-Nijmegen. The study was conducted in accordance with the principles of the most recent version of the Declaration of Helsinki and the International Conference on Harmonisation guidelines on Good Clinical Practice. Written informed consent was provided by all the participants. All authors take responsibility for the accuracy and completeness of the reported data and vouch for the fidelity of the trial to the protocol (available with the full text of this article at NEJM.org) and statistical analysis plan (which is included in the protocol). Details of the protocol and study design have been published.
previously\textsuperscript{11}. The trial was performed at two sites in the Netherlands (Radboud University Medical Center and Sint Maartenskliniek) and was overseen by an independent external data and safety monitoring board.

**STUDY POPULATION**

Patients were recruited from October 2010 through June 2013. Eligibility was assessed according to previously described inclusion and exclusion criteria (Table S1 in the Supplementary Appendix, available at NEJM.org)\textsuperscript{11}. In short, patients with persistent symptoms attributed to Lyme disease (musculoskeletal pain, arthritis, arthralgia, neuralgia, sensory disturbances, dysesthesia, neuropsychological disorders, or cognitive disorders, with or without persistent fatigue) were eligible if these symptoms either were temporally related to an erythema migrans rash or an otherwise proven case of symptomatic Lyme disease, or were accompanied by \textit{B. burgdorferi} IgG or IgM antibodies, as confirmed by means of immunoblot assay.

**RANDOMIZATION AND BLINDING**

Patients were randomly assigned to one of three groups in a 1:1:1 ratio. Randomization was computerized and balanced by minimization for age (<40 or ≥ 40 years), sex, duration of symptoms (<1 or ≥ 1 year), and baseline Global Health Composite score of the RAND-36 Health Status Inventory (RAND SF-36)\textsuperscript{12}. The randomization list consisted of consecutive medication numbers entered into a secured Web-based database by an independent Web manager. All personnel involved in the study (except the Web manager and study pharmacist) and all participants were unaware of the study-group assignments.

**INTERVENTION**

All patients received treatment with 2000 mg of open-label intravenous ceftriaxone 2000 mg daily for 14 days. Patients were admitted at the study site for ceftriaxone administration during days 1 and 2; subsequent doses were given intravenously by specialized home-care nurses. After the 2-week course of ceftriaxone treatment was completed, the patients received a 12-week oral course of doxycycline (100 mg of doxycycline twice daily combined with a placebo twice daily), clarithromycin-hydroxychloroquine (500 mg clarithromycin twice daily combined with 200 mg of hydroxychloroquine twice daily), or placebo (two different
placebo capsules twice daily), as randomly assigned in a blinded manner. The study drugs and placebo were prepared as capsules with an identical appearance. Active drugs were purchased as standard tablets through the hospital pharmacy department and were placed inside size 000 capsules; placebos were prepared by filling color-matched size 000 capsules with inactive microcrystalline cellulose. Adherence was verified by means of pill counts, patient diaries, and the Medication Event Monitoring System (AARDEX Group), in which microprocessors in the cap of a medication bottle electronically record each time a bottle is opened 13. The use of specific concomitant medication was prohibited during the entire study period, as described previously 11.

OUTCOME MEASURES
Outcomes were assessed with the use of self-completed questionnaires at baseline, at the end of the treatment period at 14 weeks (i.e. when the 2-week course of ceftriaxone and the 12-week randomized phase had been completed), at 26 weeks (12 weeks after the end of the treatment period), and at 40 weeks (the end of the trial, 26 weeks after the end of the treatment period), and at 52 weeks after the start of the treatment period. Study visits to evaluate safety were scheduled at weeks 2, 8, and 14 and included a medical history, physical examination, and laboratory investigations. The primary outcome measure was health-related quality of life at the end of the treatment period, as assessed by the physical-component summary score of the RAND SF-36 12,14. This score is based on the weighted T-scores of the four physical scales of the RAND SF-36 (physical functioning, role limitations due to physical health problems, pain, and general health perceptions). The raw SF-36 physical-component summary score was transformed into a norm-based T-score (range, 15 to 61), with a mean (±SD) score of 50±10 in the general population (higher scores indicate a better physical quality of life).

Main secondary outcomes were physical and mental aspects of health-related quality of life, as assessed with the use of the RAND SF-36 11, and fatigue, as assessed with the use of the fatigue-severity scale of the Checklist Individual Strength, on which scores range from 8 to 56, with higher scores indicating more fatigue 15 (Table 1).
STATISTICAL ANALYSIS

The primary analyses were performed in the modified intention-to-treat population, which included all patients who were randomly assigned to a study group and received at least one dose of ceftriaxone. In the primary analysis, the three study groups were compared at end of the treatment period by means of analysis of covariance, with sex and baseline SF-36 physical-component summary score as covariates. Missing data were imputed according to the baseline-value-carried-forward method. In secondary analyses, linear mixed models were used to evaluate the duration of the treatment effect in an explorative way, and missing data were imputed with the nearest available observation. All models included the baseline value of the dependent variable, sex, time, study-group assignment, and time-by-treatment interaction. No interim efficacy analysis was performed. Sensitivity analyses included a prespecified per-protocol analysis and alternative imputation techniques. Patients who had major protocol violations, such as receipt of less than 75% of a study drug or placebo, as recorded by microprocessors in the Medication Event Monitoring System caps, or use of prohibited concomitant medication, were excluded from the per-protocol analysis 11.

A two-sided alpha level of 5% was used to indicate statistical significance, and confidence intervals, when calculated, were 95% confidence intervals. Bonferroni correction was used for pairwise comparisons among the three study groups. Statistical analyses were performed with the use of SPSS software, version 20 (SPSS).

The calculation of power was based on a pilot study that included 80 patients with persistent symptoms attributed to Lyme disease 11. Patients were classified as having a poor or reasonable clinical condition, as assessed during the first clinical consultation at the outpatient clinic. The difference in SF-36 physical-component summary score between patients with a poor clinical condition and those with a reasonable clinical condition was a mean of 3±8 points, which corresponds with to the minimal clinically important difference of 2 to 5 points that has been proposed for the SF-36 physical-component summary score 14. We calculated that a minimum of 75 patients would need to be assigned to each group (225 patients in total) for the study to have 90% power to detect a difference of 3 points at a two-sided alpha of 5% and a reliability coefficient (correlation between consecutive measurements) of 0.7 16. To compensate for possible loss to follow-up, a study population of at least 255 patients was targeted.
FIGURE 1. Enrollment, randomization, and analysis.

Some patients were excluded from the per-protocol analysis because of two or more reasons. Premature discontinuation was defined as discontinuation of the study drug or placebo 7 days or more before the scheduled end of the treatment period, as recorded by microprocessors in the Medication Event Monitoring System caps that were used to track adherence. Week 14 was the end of the treatment period, after the 2-week course of ceftriaxone and the 12-week course of the randomized study drug or placebo had been completed. SF-36 denotes RAND-36 Health Status Inventory.
### Table 1. Baseline characteristics in the modified intention-to-treat population.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Doxycycline group (N = 86)</th>
<th>Clarithromycin–hydroxychloroquine group (N = 96)</th>
<th>Placebo group (N = 98)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female sex – no. (%)</strong></td>
<td>40 (47)</td>
<td>42 (44)</td>
<td>47 (48)</td>
</tr>
<tr>
<td><strong>Age – yr</strong></td>
<td>48.1 ± 12.8</td>
<td>48.2 ± 13</td>
<td>50 ± 9.7</td>
</tr>
<tr>
<td><strong>White race – no. (%)†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Current symptoms – no. (%)‡</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>80 (93)</td>
<td>87 (91)</td>
<td>84 (86)</td>
</tr>
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<td>72 (84)</td>
<td>77 (80)</td>
<td>76 (78)</td>
</tr>
<tr>
<td>Sensory disturbances</td>
<td>62 (72)</td>
<td>72 (75)</td>
<td>79 (81)</td>
</tr>
<tr>
<td>Neuralgia</td>
<td>7 (8)</td>
<td>16 (17)</td>
<td>18 (18)</td>
</tr>
<tr>
<td>Neurocognitive symptoms</td>
<td>76 (88)</td>
<td>81 (84)</td>
<td>85 (87)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>84 (98)</td>
<td>91 (95)</td>
<td>92 (94)</td>
</tr>
<tr>
<td><strong>Duration of symptoms – yr</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.7</td>
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<td>2.1</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>1.3 – 7.7</td>
<td>1.3 – 5.4</td>
<td>0.9 – 5.5</td>
</tr>
<tr>
<td><strong>Lyme disease history – no. (%)¶</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Tick bite</td>
<td>47 (55)</td>
<td>46 (48)</td>
<td>60 (61)</td>
</tr>
<tr>
<td>Erythema migrans§</td>
<td>25 (29)</td>
<td>26 (27)</td>
<td>27 (28)</td>
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<td>Acrodermatitis chronica atrophicans¶</td>
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<td>1 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Meningoradiculitis¶</td>
<td>1 (1)</td>
<td>9 (9)</td>
<td>5 (5)</td>
</tr>
<tr>
<td><strong>Previous antibiotic treatment - no. (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>40</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>27 – 57</td>
<td>21 – 44</td>
<td>28 – 58</td>
</tr>
<tr>
<td><strong>No. of courses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>1.0 – 2.0</td>
<td>1.0 – 2.0</td>
<td>1.0 – 2.5</td>
</tr>
<tr>
<td>Intravenous treatment – no. (%)</td>
<td>11 (13)</td>
<td>16 (17)</td>
<td>15 (15)</td>
</tr>
<tr>
<td><strong>Positive B. Burgdorferi serology – no. (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>70 (81)</td>
<td>73 (76)</td>
<td>75 (77)</td>
</tr>
<tr>
<td><strong>IgM</strong></td>
<td>25 (29)</td>
<td>21 (22)</td>
<td>35 (36)</td>
</tr>
<tr>
<td><strong>IgG</strong></td>
<td>55 (64)</td>
<td>65 (68)</td>
<td>58 (59)</td>
</tr>
<tr>
<td><strong>RAND SF-36 score</strong>**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical component summary</td>
<td>30.3 ± 6.3</td>
<td>32.7 ± 7.5</td>
<td>31.8 ± 8.1</td>
</tr>
<tr>
<td>Mental component summary</td>
<td>37.4 ± 9.9</td>
<td>37.1 ± 9.8</td>
<td>37.6 ± 9.6</td>
</tr>
</tbody>
</table>
Table 1. (Continued)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global health composite</td>
<td>32.1 ± 8.0</td>
<td>33.1 ± 8.3</td>
<td>33.0 ± 9.1</td>
</tr>
<tr>
<td>Physical functioning scale</td>
<td>37.3 ± 8.2</td>
<td>40.3 ± 9.9</td>
<td>38.1 ± 9.4</td>
</tr>
<tr>
<td>Role-physical scale</td>
<td>28.8 ± 5.9</td>
<td>31.3 ± 9.5</td>
<td>30.3 ± 8.6</td>
</tr>
<tr>
<td>Bodily pain scale</td>
<td>35.2 ± 8.3</td>
<td>37.3 ± 8.2</td>
<td>38.1 ± 9.4</td>
</tr>
<tr>
<td>General health scale</td>
<td>35.5 ± 7.7</td>
<td>35.9 ± 7.6</td>
<td>35.9 ± 8.4</td>
</tr>
<tr>
<td>Mental health scale</td>
<td>44.2 ± 9.8</td>
<td>43.6 ± 10.0</td>
<td>44.0 ± 8.5</td>
</tr>
<tr>
<td>Role-emotional scale</td>
<td>41.8 ± 15.1</td>
<td>39.9 ± 15.2</td>
<td>42.4 ± 14.8</td>
</tr>
<tr>
<td>Social functioning scale</td>
<td>33.5 ± 12.8</td>
<td>33.8 ± 12.0</td>
<td>34.2 ± 12.2</td>
</tr>
<tr>
<td>Vitality scale</td>
<td>38.3 ± 7.1</td>
<td>39.0 ± 7.8</td>
<td>38.3 ± 7.7</td>
</tr>
<tr>
<td>Checklist Individual Strength††</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>101.9 ± 19.4</td>
<td>96.5 ± 20.7</td>
<td>99.3 ± 22.3</td>
</tr>
<tr>
<td>Fatigue-severity scale</td>
<td>46.0 ± 8.1</td>
<td>42.7 ± 10.7</td>
<td>43.8 ± 10.6</td>
</tr>
</tbody>
</table>

* Plus–minus values are means ±SD. All study groups received a 2-week course of ceftriaxone before the randomized 12-week course of study drug or placebo. The modified intention-to-treat population included all patients who were randomly assigned to a study group and received at least one dose of ceftriaxone. Between-group differences in characteristics were analyzed with the use of analysis of variance for continuous variables, chi-square tests for proportions, and Fisher’s exact test for small numbers (expected frequency <5). Data that were not normally distributed were analyzed with the use of Kruskal–Wallis tests. There were no significant baseline differences among the study groups at a significance level of 0.05. RAND SF-36 denotes the RAND-36 Health Status Inventory.

† Race was self-reported.

‡ Categories are not mutually exclusive.

§ The condition was considered to be temporally related if it was diagnosed by a physician 0 to 4 months before the onset of symptoms.

¶ This condition was considered to be temporally related if it was diagnosed by a physician or biopsy 0 to 4 months before the onset of symptoms.

‖ The condition was considered to be temporally related if it was diagnosed on the basis of intrathecal borrelia antibody production 0 to 4 months before the onset of symptoms.

** The ranges of the RAND SF-36 scores were as follows: physical-component summary, 15 to 61; mental-component summary, 11 to 66; global-health composite, 8 to 65; physical-functioning scale, 16 to 58; role-physical scale, 26 to 56; bodily pain scale, 20 to 60; general-health scale, 20 to 64; mental-health scale, 16 to 66; role-emotional scale, 19 to 54; social-functioning scale, 12 to 57; and vitality scale, 26 to 70. For all scales, higher scores indicate better quality of life.

†† Scores on the Checklist Individual Strength range from 20 to 140 for the total score and from 8 to 56 for the fatigue-severity scale. For both scales, higher scores indicate more fatigue.
RESULTS

STUDY POPULATION AND BASELINE CHARACTERISTICS
Approximately 1200 patients were screened. The most frequent reasons for ineligibility were negative serologic findings combined with Lyme disease that was either unproven or temporally unrelated to symptoms, a coexisting condition that could account for the patient’s symptoms, or known unacceptable side effects form the active study drugs. Of all eligible patients, fewer than 10% declined to participate. A total of 281 patients underwent randomization, and 280 started the oral course of the study drug or placebo (Fig. 1). Table 1 shows the baseline characteristics of patients included in the modified intention-to-treat analysis; there were no significant baseline differences among the study groups. The randomized oral regimen (active study drug or placebo) was completed by 252 patients (90.0%): 76 of 86 patients (88.4%) in the doxycycline group, 84 of 96 patients (87.5%) in the clarithromycin-hydroxychloroquine group, and 92 of 98 patients (93.9%) in the placebo group (P = 0.28) (Fig. 1).

No difference in adherence were recorded among the study groups (P = 0.50); 75 patients (87.2%) in the doxycycline group, 78 (81.3%) in the clarithromycin-hydroxychloroquine group, and 84 (85.7%) in the placebo group took at least 75% of the assigned study medication or placebo, as recorded by the microprocessors on the Medication Event Monitoring System caps (Fig. 1).

OUTCOMES
The primary outcome in the modified intention-to-treat analysis (i.e., the mean health-related quality of life at the end of the treatment period, as indicated by the SF-36 physical-component summary score), corrected for baseline SF-36 physical-component summary score and sex), did not differ significantly among the study groups (P = 0.69) (Table 2). With respect to the secondary outcomes, the mean SF-36 physical-component summary score among all patients in the modified intention-to-treat analysis increased from 31.8 at baseline to 36.4 at the end of the treatment period (difference, 4.6 points; 95% confidence interval [CI], 3.6 to 5.5; P<0.001). At weeks 26, 40, and 52, the SF-36 physical-component summary score remained higher than the baseline score but did not change significantly from the score at the end of the treatment period in any of the study groups (Fig. 2). None of the
secondary outcome measures at the end of the treatment period differed significantly among the study groups (Table 2). Mixed-model analyses did not show any additional longer-term treatment effect with respect to the SF-36 physical-component summary score or any of the secondary outcomes; P values for time-by-treatment interaction ranged from 0.14 to 0.90, and there was no significant difference among the study groups in the SF-36 physical-component summary score (P = 0.35) or any other secondary outcome measure at any time point during follow-up. All sensitivity analyses yielded results similar to those of the main analyses. Specifically, the results were not quantitatively different when alternate imputation techniques were used for missing data (Table S4 in the Supplementary Appendix). The per-protocol analysis, which included 212 patients (Fig. 1), yielded similar results to the modified intention-to-treat analysis at the end of the treatment period and during follow-up across the three study groups.

SAFETY

Overall, 205 patients (73.2%) reported at least one adverse event, 9 patients (3.2%) had a serious adverse event, and 19 patients (6.8%) had an adverse event that led to discontinuation of the study drug (Table 3). Most adverse events were grade 1 or 2 according to the criteria of the AIDS Clinical Trials Group for grading the severity of adverse events among adults (Table S3 in the Supplementary Appendix).

During the 2-week open-label ceftriaxone phase, 131 patients (46.8%) reported at least one adverse event. Most of these adverse events were judged to be drug-related, and rash and diarrhea were the most common events. No catheter-associated infections were reported. In 6 patients, an allergic adverse event led to the discontinuation of ceftriaxone. Five serious adverse events were reported, 4 of which were allergic reactions related to ceftriaxone use.
Table 2. Treatment effect at end of the treatment period in the modified intention-to-treat population.*

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Doxycycline group (N = 86)</th>
<th>Clarithromycin–hydroxychloroquine group (N = 96)</th>
<th>Placebo group (N = 98)</th>
<th>P value†</th>
<th>Doxycycline group vs. placebo group</th>
<th>Clarithromycin–hydroxychloroquine group vs. placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-36 physical-component summary§</td>
<td>35.0 (33.5 to 36.5)</td>
<td>35.6 (34.2 to 37.1)</td>
<td>34.8 (33.4 to 36.2)</td>
<td>0.69</td>
<td>0.2 (-2.4 to 2.8)</td>
<td>0.9 (-1.6 to 3.3)</td>
</tr>
<tr>
<td><strong>Secondary outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAND SF-36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental-component summary</td>
<td>40.2 (38.6 to 41.9)</td>
<td>40.5 (38.9 to 42.1)</td>
<td>40.1 (38.6 to 41.7)</td>
<td>0.94</td>
<td>0.1 (-2.7 to 2.9)</td>
<td>0.4 (-2.3 to 3.1)</td>
</tr>
<tr>
<td>Global-health composite</td>
<td>36.1 (34.5 to 37.8)</td>
<td>36.6 (35.1 to 38.1)</td>
<td>36.0 (34.5 to 37.5)</td>
<td>0.85</td>
<td>0.1 (-2.6 to 2.9)</td>
<td>0.6 (-2.1 to 3.2)</td>
</tr>
<tr>
<td>Physical-functioning scale</td>
<td>41.9 (40.5 to 43.3)</td>
<td>42.1 (40.8 to 43.4)</td>
<td>41.0 (39.7 to 42.3)</td>
<td>0.44</td>
<td>0.9 (-1.4 to 3.2)</td>
<td>1.1 (-1.1 to 3.4)</td>
</tr>
<tr>
<td>Role-physical scale</td>
<td>33.6 (31.6 to 35.6)</td>
<td>34.4 (32.5 to 36.3)</td>
<td>33.9 (32.0 to 35.8)</td>
<td>0.84</td>
<td>-0.3 (-3.7 to 3.1)</td>
<td>0.5 (-2.8 to 3.8)</td>
</tr>
<tr>
<td>Bodily pain scale</td>
<td>39.1 (37.5 to 40.7)</td>
<td>40.5 (39.0 to 41.9)</td>
<td>39.4 (37.9 to 40.9)</td>
<td>0.42</td>
<td>-0.3 (-2.9 to 2.4)</td>
<td>1.1 (-1.5 to 3.6)</td>
</tr>
<tr>
<td>General-health scale</td>
<td>37.1 (35.6 to 38.6)</td>
<td>38.4 (37.0 to 39.8)</td>
<td>37.5 (36.2 to 38.9)</td>
<td>0.41</td>
<td>-0.4 (-2.9 to 2.0)</td>
<td>0.9 (-1.5 to 3.3)</td>
</tr>
<tr>
<td>Mental-health scale</td>
<td>45.1 (43.8 to 46.4)</td>
<td>45.2 (43.9 to 46.4)</td>
<td>45.1 (43.9 to 46.4)</td>
<td>1.00</td>
<td>0.0 (-2.3 to 2.2)</td>
<td>0.0 (-2.1 to 2.2)</td>
</tr>
<tr>
<td>Role-emotional scale</td>
<td>44.7 (42.4 to 47.0)</td>
<td>41.4 (39.2 to 43.6)</td>
<td>42.6 (40.4 to 44.8)</td>
<td>0.11</td>
<td>2.1 (-1.7 to 6.0)</td>
<td>-1.2 (-5.0 to 2.6)</td>
</tr>
<tr>
<td>Social-functioning scale</td>
<td>36.3 (34.2 to 38.4)</td>
<td>38.5 (36.6 to 40.5)</td>
<td>37.5 (35.6 to 39.5)</td>
<td>0.32</td>
<td>-1.2 (-4.7 to 2.3)</td>
<td>1.0 (-2.4 to 4.4)</td>
</tr>
<tr>
<td>Vitality scale</td>
<td>42.5 (40.9 to 44.0)</td>
<td>42.4 (41.0 to 43.9)</td>
<td>41.9 (40.5 to 43.4)</td>
<td>0.85</td>
<td>0.5 (-2.0 to 3.1)</td>
<td>0.5 (-2.0 to 3.0)</td>
</tr>
<tr>
<td>Checklist Individual Strength§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>88.7 (84.4 to 92.9)</td>
<td>87.1 (83.0 to 91.1)</td>
<td>88.4 (84.4 to 92.4)</td>
<td>0.84</td>
<td>0.3 (-6.9 to 7.4)</td>
<td>-1.3 (-8.3 to 5.6)</td>
</tr>
<tr>
<td>Fatigue-severity scale</td>
<td>39.4 (37.3 to 41.5)</td>
<td>38.6 (36.9 to 40.5)</td>
<td>38.3 (36.3 to 40.2)</td>
<td>0.73</td>
<td>1.1 (-2.4 to 4.6)</td>
<td>0.3 (-3.1 to 3.7)</td>
</tr>
</tbody>
</table>

* All study groups first received a 2-week course of ceftriaxone before the randomized 12-week course of study drug or placebo. P values were derived by analysis of covariance. All scores are adjusted for sex and baseline SF-36 physical-component summary score.
† Bonferroni correction was used for pairwise comparisons among the three study groups.
‡ Group differences should exceed 2 to 4 T-points (exact number of points varies for each scale) to indicate minimally important differences on all RAND SF-36 scales.
§ The ranges of the RAND SF-36 scores were as follows: RAND SF-36 physical-component summary, 15 to 61; mental-component summary, 11 to 66; global-health composite, 8 to 65; physical-functioning scale, 16 to 58; role-physical scale, 26 to 56; bodily pain scale, 20 to 60; general-health scale, 20 to 64; mental-health scale, 16 to 66; role-emotional scale, 19 to 54; social-functioning scale, 12 to 57; and vitality scale, 26 to 70. For all scales, higher scores indicate better quality of life.
¶ Scores on the Checklist Individual Strength range from 20 to 140 for the total score and from 8 to 56 for the fatigue-severity scale. For both scales, higher scores indicate more fatigue.
During the 12-week randomized phase, 134 patients (47.9%) had at least one adverse event (Table 3), most of which were judged to be drug-related. The percentage of patients with adverse events from any cause and with drug-related adverse events did not differ significantly among the study groups (P = 0.27 and P = 0.14, respectively). Photosensitivity and nausea were the most common events in the doxycycline group. Nausea and diarrhea
were the most common events in the clarithromycin-hydroxychloroquine group, and rash was significantly more prevalent in that group than in either of the other two groups (P = 0.01). Fourteen patients (5.0%) discontinued the randomized active drug or placebo because of an adverse event; the number of patients who discontinued their assigned regimen did not differ significantly among the three study groups (P = 0.49). Four serious adverse events were reported, none of which were drug-related.

Table 3. Adverse events in the modified intention-to-treat population.*

<table>
<thead>
<tr>
<th>Type of event</th>
<th>Total (N=280)</th>
<th>Open-label phase (N=280)</th>
<th>Doxycycline group (N=86)</th>
<th>Clarithromycin-hydroxychloroquine group (N=96)</th>
<th>Placebo group (N=98)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. of participants (percent)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any adverse event†</td>
<td>205 (73.2)</td>
<td>131 (46.8)</td>
<td>47 (54.7)</td>
<td>45 (46.9)</td>
<td>42 (42.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>Any drug-related adverse event†</td>
<td>192 (68.6)</td>
<td>127 (45.4)</td>
<td>42 (48.8)</td>
<td>42 (43.8)</td>
<td>34 (34.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>Discontinued treatment owing to adverse event‡</td>
<td>19 (6.8)</td>
<td>6 (2.1)</td>
<td>3 (3.5)</td>
<td>7 (7.3)</td>
<td>4 (4.1)</td>
<td>0.49</td>
</tr>
<tr>
<td>Any serious adverse event</td>
<td>9 (3.2)</td>
<td>5 (1.8)</td>
<td>3 (3.5)</td>
<td>1 (1.0)</td>
<td>0 (0.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Most common adverse events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>91 (32.5)</td>
<td>72 (25.7)</td>
<td>4 (4.7)</td>
<td>9 (9.4)</td>
<td>6 (6.1)</td>
<td>0.43</td>
</tr>
<tr>
<td>Nausea</td>
<td>44 (15.7)</td>
<td>20 (7.1)</td>
<td>9 (10.5)</td>
<td>10 (10.4)</td>
<td>5 (5.1)</td>
<td>0.31</td>
</tr>
<tr>
<td>Rash‡</td>
<td>31 (11.1)</td>
<td>23 (8.2)</td>
<td>1 (1.2)</td>
<td>8 (8.3)</td>
<td>1 (1.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Mucosal fungal infection</td>
<td>20 (7.1)</td>
<td>8 (2.9)</td>
<td>5 (5.8)</td>
<td>4 (4.2)</td>
<td>3 (3.1)</td>
<td>0.66</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>19 (6.8)</td>
<td>2 (0.7)</td>
<td>16 (18.6)</td>
<td>0 (0.0)</td>
<td>1 (1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Headache</td>
<td>16 (5.7)</td>
<td>12 (4.3)</td>
<td>0 (0.0)</td>
<td>2 (2.1)</td>
<td>2 (2.0)</td>
<td>0.55</td>
</tr>
<tr>
<td>Dizziness</td>
<td>16 (5.7)</td>
<td>3 (1.1)</td>
<td>3 (3.5)</td>
<td>5 (5.2)</td>
<td>5 (5.1)</td>
<td>0.88</td>
</tr>
<tr>
<td>Visual impairment</td>
<td>16 (5.7)</td>
<td>1 (0.4)</td>
<td>1 (1.2)</td>
<td>4 (4.2)</td>
<td>10 (10.2)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Data are the number of patients who had at least one event of a given type (% of study group). All patients received a 2-week course of ceftriaxone treatment (open-label phase), after which patients were randomly assigned to receive a 12-week oral course of doxycycline, clarithromycin-hydroxychloroquine, or placebo (randomized phase).

† The total is not a sum of the two trial phases because some patients had an adverse event during both phases. P values were derived from the chi-square test for the comparisons of the three study groups during the randomized phase.

‡ Fisher’s exact test was used when the numbers were small (expected frequency <5).
DISCUSSION

In this randomized, double-blind trial involving patients with persistent symptoms attributed to Lyme disease, prolonged antibiotic treatment (ceftriaxone followed by 12 weeks of either doxycycline or clarithromycin-hydroxychloroquine) did not lead to a better health-related quality of life than with shorter-term treatment (ceftriaxone followed by placebo). Patients with persistent symptoms attributed to Lyme disease have a poor quality of life, as has been reported in previous studies \(^5,6,17,18\); the low baseline RAND SF-36 scores of the patients in our trial also reflect the poor quality of life among these patients. At the 14-week study visit at the end of the treatment period, the mean SF-36 physical-component summary score had improved significantly from baseline regardless of the study-group assignment, but quality of life remained below that of the general population. Similar improvements over time, regardless of study-group assignment were reported by Kaplan et al., who compared placebo with ceftriaxone followed by doxycycline for persistent symptoms attributed to Lyme disease \(^19\).

Whether improvement in the SF-36 physical component summary score at end of treatment is a beneficial effect of shorter-term antibiotic therapy or a nonspecific effect caused by the low level of baseline functioning, expectations associated with treatment, or placebo effects remains unclear, because all the patients had received 2 weeks of open-label antibiotics before entering into the longer-term randomized study-drug or placebo phase. No significant differences among the study groups were found for any of the secondary outcomes at the end of the treatment period. In addition, no significant changes over time were observed during the 26-week follow-up after the end of the treatment period in any of the study groups.

Although we did not find a significant benefit of longer-term antibiotic therapy, we did find that there were side effects from the use of antibiotics; however, these side effects were similar among the study groups. The majority of patients (68.6%) reported a drug-related adverse event. During the open-label ceftriaxone phase, the incidence of serious adverse events was low; no patient had a serious adverse event related to the use of catheters, and 4 of 280 patients (1.4%) had allergic reactions. During the randomized phase, photosensitivity related to doxycycline use and clarithromycin-hydroxychloroquine use were the most common adverse events, and no serious adverse event was thought to be related to the randomized study drugs or placebo.
Specific efforts were made to ensure that the patients adhered to the study regimens. Using the Medication Event Monitoring System caps, we recorded that 22 patients (7.9%) discontinued treatment 7 days or more before the end of the treatment period at week 14. In a sensitivity analysis that included the 212 patients who were more than 75% adherent to the study regimen, as determined by electronic medication bottle caps, and had no major protocol violations, no significant difference was shown among the study groups.

The findings of the current trial contribute to the findings of prior work. Our results are consistent with those from the randomized, placebo-controlled trials by Klempner et al., who did not identify a benefit from treatment with ceftriaxone followed by doxycycline for a total of 90 days. However, these trials have been performed in North America, and Lyme disease in Europe is caused by different *Borrelia* species. The trials by Klempner et al. have been the subject of divergent opinions because they were discontinued prematurely after an interim analysis had indicated that a significant difference in efficacy was unlikely to be reached. Therefore, although the results are statistically valid, the value of prolonged antibiotic therapy for patients with Lyme disease has been based on a study population of about 115 patients. Others have suggested that the trials by Klempner et al. were underpowered as a result of an optimistic estimate of the size of the treatment effect. In a pilot study, we determined that the clinically relevant treatment effect on the SF-36 physical-component summary score scale was 3 points, as was recommended by the SF-36 Health Survey. None of the differences among the study groups were found to exceed the minimal clinically relevant difference for each of the RAND SF-36 scales, which varies between 2 and 4 across scales. Whereas earlier trials might have been influenced by baseline differences, we included baseline health-related quality of life as a covariate.

Three other small, placebo-controlled trials have addressed prolonged treatment for persistent symptoms attributed to Lyme disease and showed positive effects for some outcomes only. Krupp et al. reported a significant treatment effect of ceftriaxone on fatigue, but not on cognitive function, at follow-up. Fallon et al. found a beneficial effect of ceftriaxone on neurocognitive performance at week 12, but the effect was not sustained to week 24. Cameron et al. reported beneficial effects of amoxicillin on mental health scores but not on physical health, in a subgroup of patients. Although several non-comparative, open-label studies have shown beneficial effects of prolonged antimicrobial treatment, including the regimens used in the current study, randomized, controlled trials of prolonged antimicrobial treatment have not confirmed those effects.
The current trial has several limitations. First, patients received open-label antibiotics for 2 weeks before the randomized phase. Consequently, the study was designed to compare longer-term therapy with shorter-term therapy, rather than with placebo as was done in previous trials \(^{4,5,18}\). Although we did not identify any benefit of longer-term therapy, the question of whether a 2-week regimen of antibiotics is superior to withholding any therapy in our patient population remains unanswered. We chose not to include a study group that received only placebo because it was judged to be unethical to withhold treatment from patients who might have an infection at baseline that had not yet been treated. We selected ceftriaxone because it is considered the treatment of choice for disseminated Lyme disease \(^{5,8}\). Thus, although 14 weeks of antimicrobial therapy did not provide a clinical benefit for patients with persistent symptoms attributed to Lyme disease, our results cannot show whether our study may have included patients with undiagnosed active \(B. burgdorferi\) infection, who have benefited from ceftriaxone treatment.

This trial, as well as previous trials \(^{4–6,18}\), was aimed at the treatment of patients with persistent, notably distressing or impairing symptoms that emerged after well-documented Lyme disease. We acknowledge that the cause of these persistent symptoms is unclear and that these patients may be heterogeneous with respect to the pathogenesis or the duration and severity of the symptoms - which reflects the heterogeneity of the population seen in clinical practice. We prevented an imbalance in baseline characteristics among the study groups by performing a randomization balanced for duration of symptoms (< or ≥ 1 year) and baseline RAND SF-36 score. Finally, it may be argued that 14 weeks of treatment is insufficient to show a beneficial treatment effect. However, whereas prolonged antimicrobial treatment is not uncommon for various infectious diseases \(^{25,26}\), the purpose of prolonged therapy for such diseases is for the prevention of microbiological relapse rather than for a delayed onset of clinical alleviation of signs or symptoms. We are not aware of any infectious disease in which the initial effect on signs, symptoms, and laboratory findings is delayed beyond the first 3 months of effective therapy.

In conclusion, the current trial suggests that 14 weeks of antimicrobial therapy does not provide clinical benefit beyond that with shorter-term treatment among patients who present with fatigue or musculoskeletal, neuropsychological, or cognitive disorders that are temporally related to prior Lyme disease or accompanied by positive \(B. burgdorferi\) serologic findings.
ACKNOWLEDGEMENTS

We thank the data and safety monitoring board members, George E. Griffin, M.D. (University of London), Joanna in ’t Hout, M.Sc. (Radboud University, Nijmegen), and Peterhans J. van den Broek, M.D. (Leiden University), as well as Stephan Keijmel, M.D., Daniëlle van den Berg, M.D., Hans Groenewoud, M.Sc., Angela Colbers, M.Sc., Hanneke Geurts, M.Sc., and the study pharmacists at Radboud University Medical Center and Sint Maartenskliniek for their assistance.
REFERENCES

**Supplementary Table S1. Inclusion and exclusion criteria**

**Inclusion criteria**

1. Males or non-pregnant, non-lactating females who are 18 years or older
2. Complaints of musculoskeletal pain, arthritis, arthralgia, neuralgia, sensory disturbances (such as paresthesias or dysesthesias), or neuropsychological/cognitive disorders, with or without persistent fatigue, that are:
   A. either temporally related to an episode of erythema migrans or otherwise proven symptomatic Lyme borreliosis (defined as within 4 months after erythema migrans as assessed by a physician, or positive biopsy PCR or culture, or intrathecal B. burgdorferi antibody production)
   B. or accompanied by a positive B. burgdorferi IgG or IgM immunoblot (as defined by strict criteria in line with the European Union Concerted Action on Lyme Borreliosis (EUCALB) and the manufacturer of the immunoblot\(^1,2,3\)), regardless of prior ELISA IgG/IgM screening results
3. Subjects must sign a written informed consent form

**Exclusion criteria**

1. Subjects with a known history of allergy or intolerance to tetracyclines, macrolides, hydroxychloroquine, or ceftriaxone
2. Subjects who have had more than 5 days of antimicrobial therapy with activity against B. burgdorferi within the previous 4 weeks
3. Subjects with a presumed diagnosis of neuroborreliosis (CSF pleiocytosis or intrathecal antibody production) for which intravenous antimicrobial therapy is required
4. Subjects with a known diagnosis of HIV-seropositivity or other immune disorders
5. Subjects with positive syphilis serology or signs of other spirochetal diseases
6. Subjects with moderate or severe liver disease defined as ALP, ALT, or AST greater than 3 times upper limit of normal
7. Subjects who are receiving and cannot discontinue cisapride, astemizole, terfenadine, barbiturates, phenytoin, or carbamazepine
8. Subjects who are currently enrolled on other investigational drug trials or receiving investigational agents
9. Subjects who have been previously randomized into this study
10. Severe physical or psychiatric co-morbidity that interferes with participation in the study protocol, including previous medical diagnosis of rheumatic conditions, chronic fatigue syndrome, or chronic pain conditions, as well as insufficient command of the Dutch language
11. Co-morbidity that could (partially) account for the symptoms of the subject (e.g., vitamin B12 deficiency, anemia, hypothyroidism)
12. Subjects of child-bearing potential unwilling to use contraception methods other than oral contraceptives during the study therapy period

**Abbreviations:**
- PCR, polymerase chain reaction
- CSF, cerebrospinal fluid
- ALP, alkaline phosphatase
- ALT, alanine aminotransferase
- AST, aspartate aminotransferase


### Table S2. Serious Adverse Events

<table>
<thead>
<tr>
<th>Type of event</th>
<th>Patient (Gender, Age)</th>
<th>Treatment phase and group</th>
<th>Causality with treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worsening of pre-existing symptoms</td>
<td>F, 61</td>
<td>Open-label ceftriaxone</td>
<td>Improbable</td>
<td>Hospitalization prolongation (1 day)</td>
</tr>
<tr>
<td>Worsening of longstanding pre-existing complaints after 2nd ceftriaxone dose</td>
<td></td>
<td></td>
<td></td>
<td>Resolved.</td>
</tr>
<tr>
<td>chest pain, nausea, headache. Ceftriaxone was continued per protocol.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>F, 26</td>
<td>Open-label ceftriaxone</td>
<td>Probable</td>
<td>Hospitalization (1 day)</td>
</tr>
<tr>
<td>Fever, chills, nausea and dizziness, starting 2 hours after each ceftriaxone infusion. Skin rash beginning Day 7. Ceftriaxone discontinued</td>
<td></td>
<td></td>
<td></td>
<td>Resolved.</td>
</tr>
<tr>
<td>Type I allergic reaction</td>
<td>F, 35</td>
<td>Open-label ceftriaxone</td>
<td>Certain</td>
<td>Hospitalization (1 day)</td>
</tr>
<tr>
<td>Urticarial rash 35 minutes after first dose of ceftriaxone.</td>
<td></td>
<td></td>
<td></td>
<td>Resolved. Discontinued from study.</td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>F, 40</td>
<td>Open-label ceftriaxone</td>
<td>Possible</td>
<td>Hospitalization (1 day)</td>
</tr>
<tr>
<td>Type I allergic reaction</td>
<td>F, 41</td>
<td>Open-label ceftriaxone</td>
<td>Certain</td>
<td>Hospitalization (1 day)</td>
</tr>
<tr>
<td>Glottis edema, dyspnea, pruritus during first ceftriaxone infusion.</td>
<td></td>
<td></td>
<td></td>
<td>Resolved. Discontinued from study.</td>
</tr>
<tr>
<td>Planned hospitalization</td>
<td>M, 47</td>
<td>Randomized doxycycline</td>
<td>Not related</td>
<td>n/a</td>
</tr>
<tr>
<td>Hospitalization of two days for planned GI workup re. abdominal pain, existing prior to randomization.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal complaints</td>
<td>F, 63</td>
<td>Randomized clarithromycin/ hydroxychloroquine</td>
<td>Improbable</td>
<td>Hospitalization (1 day)</td>
</tr>
<tr>
<td>General discomfort, nausea and vomiting.</td>
<td></td>
<td></td>
<td></td>
<td>Resolved.</td>
</tr>
<tr>
<td>Chest pain due to coronary artery stenosis. Scheduled for elective percutaneous transluminal coronary angioplasty.</td>
<td>F, 59</td>
<td>Randomized doxycycline</td>
<td>Not related</td>
<td>Hospitalization (1 day)</td>
</tr>
<tr>
<td>Traffic injury</td>
<td>M, 67</td>
<td>Randomized doxycycline</td>
<td>Not related</td>
<td>Hospitalization (1 day)</td>
</tr>
<tr>
<td>Sternal fracture, 2 weeks after end of study medication.</td>
<td></td>
<td></td>
<td></td>
<td>Resolved.</td>
</tr>
</tbody>
</table>
## Supplementary Table S3. Adverse Events by type, grade, and treatment allocation

<table>
<thead>
<tr>
<th>Type of event</th>
<th>Total* (n)</th>
<th>Grade 1 (n)</th>
<th>Grade 2 (n)</th>
<th>Grade 3 (n)</th>
<th>Grade 4 (n)</th>
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</thead>
<tbody>
<tr>
<td><strong>Ceftiaxone treatment phase</strong></td>
<td></td>
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<td>Diarrhea</td>
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<tr>
<td>Nausea</td>
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<td>8</td>
<td>12</td>
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<td>0</td>
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<tr>
<td>Rash / Allergic reaction</td>
<td>23</td>
<td>7</td>
<td>14</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Mucosal fungal infection</td>
<td>9</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
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<td>3</td>
<td>9</td>
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<tr>
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<td>3</td>
<td>2</td>
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<td>0</td>
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<tr>
<td>Visual impairment</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Liver transaminase (ALT) elevation</td>
<td>39</td>
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<tr>
<td><strong>Randomized doxycycline treatment</strong></td>
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<td></td>
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<td></td>
</tr>
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<td>Diarrhea</td>
<td>4</td>
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<td>0</td>
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<tr>
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<td>9</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rash / Allergic reaction</td>
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<td>Dizziness</td>
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<td>2</td>
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</tr>
<tr>
<td>Visual impairment</td>
<td>1</td>
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<tr>
<td>Insomnia</td>
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<tr>
<td>Tooth discoloration</td>
<td>2</td>
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<td>Liver transaminase (ALT) elevation</td>
<td>1</td>
<td>1</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Randomized claritromycin/hydroxychloroquine treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Rash / Allergic reaction</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mucosal fungal infection</td>
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<td>Headache</td>
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<td>1</td>
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</tr>
<tr>
<td>Dizziness</td>
<td>4</td>
<td>3</td>
<td>1</td>
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<td>0</td>
</tr>
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<td>Visual impairment</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Insomnia</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liver transaminase (ALT) elevation</td>
<td>4</td>
<td>4</td>
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<td>0</td>
</tr>
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<td>Tooth discoloration</td>
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<td>0</td>
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<tr>
<td>Taste change</td>
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</tr>
<tr>
<td><strong>Randomized placebo treatment</strong></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rash / Allergic reaction</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mucosal fungal infection</td>
<td>3</td>
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<td>0</td>
</tr>
<tr>
<td>Photosensitivity</td>
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<td>1</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dizziness</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Visual impairment</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Insomnia</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Liver transaminase (ALT) elevation</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Number of adverse events are represented. Some patients have multiple adverse events of the same type.
† Grading according to ACTG grading severity of adult adverse events.
The primary outcome measure was health-related quality of life at end of treatment (EOT; week 14), assessed by the physical component summary score (PCS) of the RAND-36 Health Status Inventory.

Missing data were imputed by carrying the baseline value forward. In a sensitivity analysis, alternate imputation techniques have been used for missing primary endpoint observations:

1. Omitting missing data;
2. Using Generalized Least Squares (equivalent to a mixed model);
3. Alleviating the 3-weeks’ maximum window around the EOT study visit requirement, i.e., nearest neighbor imputation – excluding the baseline value for imputation;
4. Standard imputing using multiple imputation by chained equations. We imputed 40 times (as the number of missing values is limited). As predictors, we used the variables in the analysis model completed with the baseline observation on the secondary outcome variables.

Pooling was done using Rubin’s rules. None of these methods yielded different outcomes, as specified in the table below.

**Supplementary Table S4.** Sensitivity analyses of alternate imputation techniques for missing observations

<table>
<thead>
<tr>
<th>Type of sensitivity analysis</th>
<th>P-value*</th>
<th>PCS Difference, placebo vs doxycycline (95% CI)</th>
<th>PCS Difference, placebo vs clarithromycin/hydroxychloroquine (95% CI)</th>
<th>PCS Difference, doxycycline vs clarithromycin/hydroxychloroquine (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omitting Missing Data</td>
<td>0.590</td>
<td>-0.198 (-3.382; 2.986)</td>
<td>-1.240 (-4.332; 1.851)</td>
<td>-1.042 (-4.269; 2.184)</td>
</tr>
<tr>
<td>Generalized Least Squares</td>
<td>0.711</td>
<td>-0.300 (-3.476; 3.877)</td>
<td>-1.040 (-4.145; 2.065)</td>
<td>-0.741 (-3.961; 2.481)</td>
</tr>
<tr>
<td>Nearest Neighbor</td>
<td>0.567</td>
<td>-0.402 (-3.239; 2.436)</td>
<td>-1.200 (-3.956; 1.553)</td>
<td>-0.800 (-3.670; 2.070)</td>
</tr>
<tr>
<td>Multiple Imputation</td>
<td>0.686</td>
<td>-0.272 (-2.803; 2.260)</td>
<td>-0.978 (-3.464; 1.509)</td>
<td>-0.706 (-3.290; 1.878)</td>
</tr>
<tr>
<td>Baseline Carried Forward</td>
<td>0.689</td>
<td>-0.201 (-2.761; 2.358)</td>
<td>-0.855 (-3.331; 1.622)</td>
<td>-0.653 (-3.233; 1.927)</td>
</tr>
</tbody>
</table>

* Pairwise comparisons between treatment arms with Bonferroni correction for comparing 3 arms.
Longer-term therapy for symptoms attributed to Lyme disease

Bart Jan Kullberg
Anneleen Berende
Andrea W. M. Evers

N Engl J Med. 2016 Sep 8;375(10):998
TO THE EDITOR

In the placebo-controlled trial by Berende et al. (March 31 issue) involving patients with persistent symptoms attributed to Lyme disease, all the patients received an initial 2-week course of intravenous ceftriaxone. Among the patients who subsequently received placebo, was the 12.6% reduction from baseline in the fatigue score the result of the initial 2-week course of ceftriaxone? The results from other studies that have involved patients with post-treatment symptoms of Lyme disease may help answer this question. In two separate studies, the effect of an intravenous placebo on fatigue was assessed over a 6-month period with the use of an 11-item fatigue-severity scale. In one study, a 9.1% reduction from baseline in the fatigue score was observed in the placebo group, and in the second study, a 14.5% reduction from baseline was observed in the placebo group. Thus, the fact that the magnitude of reduction in fatigue score among the participants who were given placebo in other studies of post-treatment Lyme disease symptoms was similar to that observed in the trial by Berende et al. suggests that the 2-week course of ceftriaxone in this trial probably provided no therapeutic benefit with respect to fatigue.

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REFERENCES
TO THE EDITOR

The trial by Berende et al. addresses an often-discussed issue regarding longer treatment duration for persistent symptoms attributed to Lyme disease. Although the inclusion criteria and the design of the study reflect clinical practice and the results are valuable for discouraging unneeded longer-term antibiotic treatment, we would like to highlight an important limitation. The diagnosis of Lyme disease in patients who do not have the classic clinical manifestations is challenging and prone to error. In this trial, a considerable percentage of patients, in particular patients who had nonspecific symptoms and only IgM antibodies, may not have had Lyme disease. The median duration of symptoms was more than 2 years, and therefore positive IgG antibodies, not IgM antibodies, are required to confirm Lyme disease. A total of 22 to 36% of the patients received a diagnosis of Lyme disease on the basis of positive IgM antibodies, and these patients probably did not benefit from any antibiotic treatment because they had received a misdiagnosis. The inclusion of these patients may have blurred a possible difference between the placebo group and the two antibiotic treatment groups, although in our experience as well, longer treatment duration does not have an effect on the severity of symptoms.

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REFERENCE

RESPONSE

The authors reply: We agree with Wormser that any effects observed during the follow-up of our patient groups cannot be attributed to ceftriaxone, as was discussed in our article. Our trial was designed to compare longer-term therapy with shorter-term therapy and does not allow for any conclusions to be made on the potential effects of the standardized pretreatment with ceftriaxone in all randomized study groups. As Wormser suggests, the reported changes in outcomes, including fatigue severity, may be ascribed to placebo effects. Responses to placebo are known to contribute to beneficial outcomes in clinical trials, as has been shown for a broad variety of symptoms, including fatigue, and for physiological responses, such as behaviorally conditioned suppression of markers of infection and immunity. Placebo effects are based on the expectations of patients and care professionals regarding the benefits of treatment. These expectations are shaped by a combination of conscious and automatic learning processes, such as conditioning by drug use and patient–physician interactions. Investigators in future trials may disentangle these effects by using more sophisticated research designs to allow comparisons with the natural course of disease (without any placebo effects) and open-label conditions (to maximize beneficial placebo responses).

Erb et al. suggest that patients with Borrelia burgdorferi IgM antibodies may not have had Lyme disease. However, IgM antibodies are known to persist for up to 3 years after infection, and false positive IgM immunoblot results occurred in fewer than 10% of healthy controls in a recent study. Our inclusion criteria aimed at selecting patients who did not have proof of active Lyme disease at baseline but who had been infected by B. burgdorferi previously. Patients had to have either documented, proven Lyme disease diagnosed a maximum of 4 months before the onset of symptoms or serologic proof of prior infection, as confirmed by immunoblot assay. Only 25 patients (9%) were included in the trial solely on the basis of positive IgM immunoblot assay results as a marker of prior infection. Among those patients, the physical-component summary score of the RAND-36 Health Status Inventory at the end of therapy was similar to that of patients who were negative for IgM antibodies and did not differ significantly among the study groups. Sensitivity analyses that excluded patients who were positive for IgM antibodies yielded results similar to those of the main analyses. Thus, the assumptions by Erb et al. are unwarranted.
REFERENCES


Longer-term therapy for symptoms attributed to Lyme disease
Cost-effectiveness of longer-term versus shorter-term provision of antibiotics in patients with persistent symptoms attributed to Lyme disease

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ABSTRACT

Background: The treatment of persistent symptoms attributed to Lyme disease remains controversial. Recently, the PLEASE study did not demonstrate any additional clinical benefit of longer-term versus shorter-term antibiotic treatment. However, the economic impact of the antibiotic strategies has not been investigated.

Methods: This prospective economic evaluation, adhering a societal perspective, was performed alongside the PLEASE study, a multicenter, placebo-controlled, double-blind 1:1:1 randomized clinical trial in which all patients received open-label intravenous ceftriaxone for two weeks before the 12-week randomized blinded oral antibiotic regimen (doxycycline, clarithromycin plus hydroxychloroquine, or placebo). Between 2010 and 2013, patients (n=271) with borreliosis-attributed persistent symptoms were enrolled and followed for one year. Main outcomes were costs, quality-adjusted life years, and incremental net monetary benefit of longer-term versus shorter-term antibiotic therapy.

Results: Mean quality-adjusted life years (95% CI) were not significantly different (p=0.96): 0.82 (0.77-0.88) for ceftriaxone/doxycycline (n=82), 0.81 (0.76-0.88) for ceftriaxone/clarithromycin-hydroxychloroquine (n=93), and 0.81 (0.76-0.86) for ceftriaxone/placebo (n=96). Total societal costs per patient (95% CI) were not significantly different either (p=0.35): €11,995 (€8,823-€15,670) for ceftriaxone/doxycycline, €12,202 (€9,572-€15,253) for ceftriaxone/clarithromycin-hydroxychloroquine, and €15,249 (€11,294-€19,781) for ceftriaxone/placebo. Incremental net monetary benefit (95% CI) for ceftriaxone/doxycycline compared to ceftriaxone/placebo varied from €3,317 (-€2,199-€8,998) to €4,285 (-€6,085-€14,524) over the willingness-to-pay range, and that of ceftriaxone/clarithromycin-hydroxychloroquine compared to ceftriaxone/placebo from €3,098 (-€888-€7,172) to €3,710 (-€4,254-€11,651). For every willingness-to-pay threshold, the incremental net monetary benefits did not significantly differ from zero.
Conclusion: The longer-term treatments were similar with regard to costs, effectiveness and cost-effectiveness compared to shorter-term treatment in patients with borreliosis-attributed persistent symptoms after one year of follow-up. Given the results of this study, and taking into account the external costs associated with antibiotic resistance, the shorter-term treatment is the antibiotic regimen of first choice.
INTRODUCTION

Lyme borreliosis, a tick-borne disease caused by the spirochete *Borrelia burgdorferi* sensu lato complex, is the most common tick-borne disease in the northern hemisphere and its incidence has been increasing considerably in several countries worldwide. Patients in the early stages of Lyme disease can often be treated successfully with antibiotics. However, regardless of initial appropriate antibiotic treatment, persistent symptoms may develop that consist of pain, neurologic or cognitive impairments, musculoskeletal symptoms and/or fatigue.

The disease burden of Lyme disease is large, and the disability-adjusted life years (DALYs) per 100,000 population were estimated at 10.55 in 2010, resulting in 1749 DALYs for the Dutch population. Mainly persistent symptoms are a considerable source of healthcare utilization and costs. Since the incidence of Lyme disease is rising in several countries, there are concerns that the significant economic and disease burden of persistent symptoms attributed to Lyme disease will increase further.

The treatment of persistent symptoms attributed to Lyme disease remains controversial, as previous trials found inconclusive results and as clinical guidelines recommend different treatment durations. Recently, the Persistent Lyme Empiric Antibiotic Study Europe (PLEASE), which evaluated the effectiveness of longer-term versus shorter-term antibiotic treatment among patients with borreliosis-attributed persistent symptoms, did not demonstrate any additional clinical benefit of longer-term antibiotic treatment compared to shorter-term treatment.

Regardless of clinical effect, it is important to assess the economic impact of the comparative antibiotic strategies. This is essential for policy makers, in order to prioritize and making complex decisions about healthcare interventions. Therefore, we performed the first cost-utility analysis of longer-term versus shorter-term provision of antibiotics in patients with persistent symptoms attributed to Lyme disease.
MATERIALS AND METHODS

STUDY DESIGN AND PATIENTS

This economic evaluation was performed alongside the PLEASE study, a multicenter, placebo-controlled, double-blind randomized clinical trial, which was conducted in the Netherlands at the Radboud university medical center and the Sint Maartenskliniek (ClinicalTrials.gov number NCT01207739). Its design and main results have been described in detail elsewhere. Briefly, patients were included if they experienced persistent symptoms attributed to Lyme disease, such as pain, musculoskeletal symptoms, neuralgia, sensory disturbances, arthritis, arthralgia, or neuropsychological/cognitive complaints, with or without persistent fatigue. These symptoms had to be preceded by an erythema migrans (EM) or otherwise confirmed symptomatic Lyme disease, or patients were required to have B. burgdorferi IgG or IgM antibodies. After inclusion, patients were randomly allocated in a 1:1:1 ratio to three treatment arms. All patients received 2000 mg open-label intravenous ceftriaxone every day for two weeks before starting the blinded oral antibiotic regimen of 12 weeks. The randomized oral treatment consisted of 100 mg of doxycycline twice daily combined with a placebo twice daily, 500 mg clarithromycin twice daily combined with 200 mg of hydroxychloroquine twice daily, or two placebos twice daily. Ethical clearance of the PLEASE study protocol was obtained from the Medical Ethics Review Committee CMO Region Arnhem-Nijmegen and all patients provided written informed consent before inclusion.

OUTCOME MEASURES

The main outcome measures of the economic evaluation were costs and EQ-5D-based quality-adjusted life years (QALYs). These outcome measures were combined into the incremental Net Monetary Benefit (NMB), adhering a societal perspective, over a one-year follow-up period. The societal perspective includes the impact of an intervention on the welfare of the whole of society, by including not only direct health effects (both costs and QALYs) but also indirect health effects (such as productivity losses). Outcomes were assessed at baseline and at 14, 26, 40 and 52 weeks follow-up by self-completed questionnaires.
EFFECTIVENESS

The quality of the health status of the patients was measured with a validated health-related quality of life (HRQoL) instrument, the EuroQol-5D (EQ-5D)\textsuperscript{20}. This HRQoL instrument was offered in a validated Dutch translation and was completed by the patients at all evaluation moments\textsuperscript{17}. The EQ-5D is a generic HRQoL instrument comprising five domains: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The EQ-5D index is obtained by applying predetermined weights to the five domains. This index gives a societal-based global quantification of the patient’s health status on a scale ranging from zero (death) to one (perfect health). Based on the EQ-5D index scores, QALYs were determined over the total follow-up period by using the trapezium rule to estimate the area under the curve.

COSTS

The following cost categories were considered: intervention costs, healthcare utilization, pain medication utilization, travel expenses and productivity losses. Intervention costs were standardized and, for each patient, consisted of one hospital admission day for the first ceftriaxone administration, two weeks of outpatient treatment with ceftriaxone, 12 hours of home care, and 12 weeks of treatment with the randomized oral regimen. The cost analysis comprised two main parts. First, on a patient level, volumes of care were measured prospectively over the time path of the clinical trial using an adapted version of the first part of the ‘Trimbos and iMTA questionnaire on Costs associated with Psychiatric illness’ (TIC-P)\textsuperscript{21} complemented with patient out-of-pocket expenses for pain-related over-the-counter drugs. Where relevant, (missing) entries were verified or completed by data from medical records. Second, standard cost prices were determined using the Dutch guideline for cost analysis in healthcare research\textsuperscript{22} and www.medicijnkosten.nl, a website managed by the Dutch National Health Care Institute, for drug prices per daily dose. If no standardized cost prices were available, real tariffs or costs were used. Productivity losses for patients were estimated using a patient-based questionnaire (Short Form – Health and Labor Questionnaire (SF-HLQ))\textsuperscript{23}. The friction cost-method was applied in accordance with the Dutch guidelines\textsuperscript{22}. In addition, travel expenses were computed for every healthcare visit based on the patient’s postal code and the location of healthcare provision. Data on resource use were multiplied by standardized unit prices to calculate costs in the treatment groups. Costs were calculated...
in Euros using 2015 as base-year value. Prices were indexed using the Consumer Price Index (CPI) from Statistics Netherlands (CBS).

DATA ANALYSIS

Patients who had been randomized into the study and who had received at least one dose of ceftriaxone were included in the modified intention-to-treat analysis. If patients only completed baseline assessment, they were considered uninformative and therefore excluded from the economic analysis. In the base-case analysis, missing data regarding QALYs and costs were imputed with the nearest available observation as this was considered the most realistic scenario. Missing data regarding time between follow-up visits were imputed a single time using a uniform random number generator, which was limited by the minimum and maximum in the available data.

Analyses of covariance, with paid work and baseline values as covariates, were used to compare mean costs and mean QALYs gained between the three treatment groups. The cost-effectiveness analysis comprised computing NMBs for each patient by multiplying the QALYs that were gained during the one year follow-up with a range of ‘willingness-to-pay (WTP) for a QALY’ thresholds and then subtracting the total costs from this amount. Subsequently, the incremental NMBs were calculated by subtracting the mean NMB of the placebo group from the mean NMBs of the longer-term treatment arms. The NMB framework, combined with multivariable linear regression, was used to correct for relevant baseline differences (paid work), baseline EQ-5D index score and baseline total costs. Because the cost-effectiveness threshold in the Netherlands ranges from €10,000 to €80,000 per QALY depending on the disease burden, six thresholds for the maximum WTP for a QALY were applied ranging from zero to 100,000 Euros. For all regression models, 1,000 bootstrap replications were used to account for skewness of the distribution of the estimator (NMB) in order to obtain robust 95% confidence intervals of the estimates. IBM SPSS Statistics 22.0 was used as statistical software package.

IMPUTATION SCENARIO ANALYSES

To analyze the effect of the abovementioned missing data imputation strategy on our results, two other imputation scenarios for missing QALYs and costs were applied in addition to the
more realistic base-case nearest available observation imputation: as a best-case scenario EQ-5D index scores were imputed with the 75th percentile of the available data and the total costs with the 25th percentile, and as a worst-case scenario EQ-5D index scores were imputed with the 25th percentile and total costs with the 75th percentile of the available data.

RESULTS

In total, 281 patients were enrolled into the PLEASE study between October 2010 and June 2013, and followed for one year. Of these, 271 patients were included in the cost-utility analysis: one patient did not start the ceftriaxone treatment; nine other patients only underwent baseline EQ-5D assessment. Baseline characteristics were not significantly different between the study groups, except for paid work, gaining income from the Work and Income according to Labor Capacity Act (WIA), and travel expenses in the three months before the start of the study (Table 1).

The average amount of QALYs yielded during the one-year follow-up period was 0.82 (95% CI, 0.77-0.88) for the ceftriaxone plus doxycycline group, 0.81 (95% CI, 0.76-0.88) for the ceftriaxone plus clarithromycin-hydroxychloroquine group, and 0.81 (95% CI, 0.76-0.86) for the ceftriaxone plus placebo group. These were not significantly different between the groups (p=0.96). The mean total societal costs over the one-year study period were also not statistically significantly different between the groups (p=0.35): €11,995 (95% CI, €8,823-€15,670) for the ceftriaxone plus doxycycline group, €12,202 (95% CI, €9,572-€15,253) for the ceftriaxone plus clarithromycin-hydroxychloroquine group and €15,249 (95% CI, €11,294-€19,781) for the ceftriaxone plus placebo group. The incremental total costs were plotted against the incremental QALYs for each longer-term treatment group compared to the placebo group in Fig 1.
# Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Ceftriaxone + doxycycline (N = 82)</th>
<th>Ceftriaxone + clarithromycin-hydroxychloroquine (N = 93)</th>
<th>Ceftriaxone + placebo (N = 96)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age – mean (SD)</td>
<td>48.6 (12.8)</td>
<td>48.5 (13.1)</td>
<td>50.2 (9.7)</td>
<td>0.56</td>
</tr>
<tr>
<td>Female sex – no. (%)</td>
<td>38 (46)</td>
<td>41 (44)</td>
<td>46 (48)</td>
<td>0.87</td>
</tr>
<tr>
<td>Employment statusb – no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paid work</td>
<td>49 (60)</td>
<td>65 (70)</td>
<td>76 (79)</td>
<td>0.02</td>
</tr>
<tr>
<td>Unpaid work</td>
<td>7 (9)</td>
<td>6 (7)</td>
<td>7 (7)</td>
<td>0.87</td>
</tr>
<tr>
<td>Unemployed</td>
<td>4 (5)</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>0.47</td>
</tr>
<tr>
<td>Student</td>
<td>3 (4)</td>
<td>5 (5)</td>
<td>2 (2)</td>
<td>0.49</td>
</tr>
<tr>
<td>Housewife/man</td>
<td>14 (17)</td>
<td>15 (16)</td>
<td>14 (15)</td>
<td>0.90</td>
</tr>
<tr>
<td>General old-age insurance</td>
<td>11 (13)</td>
<td>8 (9)</td>
<td>8 (8)</td>
<td>0.46</td>
</tr>
<tr>
<td>Sickness Benefits Act</td>
<td>17 (21)</td>
<td>20 (22)</td>
<td>27 (28)</td>
<td>0.43</td>
</tr>
<tr>
<td>Labor disability (WIA)</td>
<td>17 (21)</td>
<td>15 (16)</td>
<td>6 (6)</td>
<td>0.02</td>
</tr>
<tr>
<td>EQ-5D index score – mean (SD)</td>
<td>0.58 (0.26)</td>
<td>0.59 (0.25)</td>
<td>0.64 (0.23)</td>
<td>0.22</td>
</tr>
<tr>
<td>Direct costs within healthcarec – mean (SD)</td>
<td>23 (39)</td>
<td>36 (89)</td>
<td>15 (26)</td>
<td>0.05</td>
</tr>
<tr>
<td>Pain medication (€)</td>
<td>7 (19)</td>
<td>5 (10)</td>
<td>6 (13)</td>
<td>0.68</td>
</tr>
<tr>
<td>Health care consumption (€)</td>
<td>497 (785)</td>
<td>1292 (5611)</td>
<td>582 (2074)</td>
<td>0.25</td>
</tr>
<tr>
<td>Direct costs outside healthcarec – mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Travel expenses (€)</td>
<td>23 (39)</td>
<td>36 (89)</td>
<td>15 (26)</td>
<td>0.05</td>
</tr>
<tr>
<td>Indirect costsd – mean (SD)</td>
<td>2544 (5535)</td>
<td>1972 (4824)</td>
<td>2710 (4660)</td>
<td>0.57</td>
</tr>
<tr>
<td>Total costsd (€) – mean (SD)</td>
<td>3072 (5770)</td>
<td>3036 (7328)</td>
<td>3314 (5078)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

* Continuous variables were compared between the groups by using an ANOVA, categorical variables by using chi-squared tests. Not normally distributed data were analyzed with a bootstrapped ANOVA.

b Categories are not mutually exclusive.

c Baseline costs are the costs in Euros made in the three months before the start of the study. WIA, Work and Income according to Labor Capacity Act.
FIGURE 1. Incremental total costs plotted against incremental QALYs with 95% confidence intervals.

FIGURE 2. Distribution of the costs of healthcare consumption in the three treatment groups, including explanation of the largest outliers.
Antibiotic therapy, productivity losses and healthcare consumption were the main cost drives in all study groups (Table 2). No significant differences in mean costs between the study arms were found in any of the cost categories, although the point estimates for healthcare consumption varied considerably (Table 2).

Fig 2 shows the distribution of healthcare consumption costs. As shown in Fig 2, a few outliers in the clarithromycin-hydroxychloroquine and placebo groups are responsible for the large distribution width. The outliers were mainly due to high costs of home adaptations (e.g. placement of an elevator). Due to bootstrapping the relevance of these outliers is relatively small and had little influence on the coefficients nor confidence intervals of the NMB regression model.

Table 2. Mean QALYs and costs (in euro’s) per patient over the 1-year follow-up period

<table>
<thead>
<tr>
<th></th>
<th>Ceftriaxone + doxycycline (N = 82)</th>
<th>Ceftriaxone + clarithromycin-hydroxychloroquine (N = 93)</th>
<th>Ceftriaxone + placebo (N = 96)</th>
<th>P value a</th>
</tr>
</thead>
<tbody>
<tr>
<td>QALYs</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Direct costs within healthcare</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic therapy b</td>
<td>2254 (-)</td>
<td>2282 (-)</td>
<td>2211 (-)</td>
<td>-</td>
</tr>
<tr>
<td>Pain medication</td>
<td>36 (23 - 52)</td>
<td>22 (16 - 29)</td>
<td>33 (22 - 45)</td>
<td>0.24</td>
</tr>
<tr>
<td>Healthcare consumption</td>
<td>1802 (1211 – 2517)</td>
<td>2324 (1508 – 3286)</td>
<td>3296 (1675 – 5521)</td>
<td>0.35</td>
</tr>
<tr>
<td>Direct costs outside healthcare</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Travel expenses</td>
<td>104 (66 - 148)</td>
<td>58 (39 - 75)</td>
<td>83 (51 - 127)</td>
<td>0.23</td>
</tr>
<tr>
<td>Indirect costs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Productivity losses</td>
<td>7667 (4466 – 12039)</td>
<td>7858 (5450 – 10667)</td>
<td>9392 (6941 – 12270)</td>
<td>0.70</td>
</tr>
<tr>
<td>Total costs</td>
<td>11995 (8823 - 15670)</td>
<td>12202 (9572 - 15253)</td>
<td>15249 (11294 - 19781)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

a Bootstrapped ANCOVA corrected for baseline value and paid work.  
b Costs of the antibiotic therapy were standardized.  
QALY, quality-adjusted life year.

Fig 3a presents the incremental Net Monetary Benefit (iNMB) with 95% CI of both longer-term treatment groups compared to the placebo group over the ‘willingness-to-pay (WTP)’ range from 0 to 100,000 Euros per QALY. The iNMB for the doxycycline group compared to the placebo group varied from €3,317 (95% CI, -€2,199–€8,998) to €4,285 (95% CI,
-€6,085-€14,524) over the WTP range, and that of the clarithromycin-hydroxychloroquine group compared to the placebo group from €3,098 (95% CI, -€888-€7,172) to €3,710 (95% CI, -€4,254-€11,651). For all WTP thresholds, the iNMBs did not significantly differ from zero. The imputation scenario analyses showed similar results (Fig 3b and 3c).

FIGURE 3. a. Base case analysis: Nearest available observation imputation. b. Best-case imputation scenario. c. Worst-case imputation scenario
DISCUSSION

This study is the first economic evaluation of antibiotic therapy regimens in patients with borreliosis-attributed persistent symptoms, and was performed alongside the PLEASE study. We found no differences in mean total societal costs and mean QALYs between the three groups after a one-year study period. There was, given the range of evaluation, no willingness-to-pay threshold for which the incremental Net Monetary Benefit of the longer-term treatment groups compared to the placebo group was significantly different from zero. The imputation scenario analysis also showed no significant differences in cost-effectiveness between the groups.

These results are relevant for the care of patients with persistent symptoms attributed to Lyme disease, as they complement the effectiveness knowledge obtained from the PLEASE study and other research. To our knowledge, no other studies have investigated the cost-utility of antibiotic treatment regimens of persistent symptoms attributed to Lyme disease. Because the PLEASE study did not show any additional clinical benefit of longer-term compared to shorter-term treatment on health-related quality of life, we did not expect to find any large differences in costs and cost-effectiveness as the oral antibiotic treatment is low-priced. Nevertheless, because of the limited resources in healthcare, cost-effectiveness of the treatment strategies should be carefully considered as one of the criteria in a rational decision-making process.

Strengths of this economic evaluation include the prospective design, in which data were collected alongside a multicenter, placebo-controlled, double-blind randomized clinical trial. This study was the largest trial that evaluated antibiotic therapies in patients with borreliosis-attributed persistent symptoms. Data on health states and costs were prospectively collected on patient-level, which made it possible to give relatively precise estimates. Moreover, we performed our analyses from a societal perspective, since we included productivity losses and travel expenses as cost categories as well. This is the optimal perspective, as it includes all relevant societal costs and benefits irrespective of who bears or accrues them, and is recommended by the Dutch guideline for economic evaluations in healthcare.

Our study also has limitations. First, there were missing data. Missing data are almost unavoidable in economic evaluations performed alongside a clinical trial, especially
when patients have to self-report the data and when costs and cost-effectiveness are not the primary outcome measures. To overcome this problem and to assess the effect of our imputation strategy, we imputed our data according to three scenarios. Since all scenarios gave slightly different estimates but similar conclusions, our results are robust against assumptions regarding the missingness of data.

Furthermore, the cause of the persistent symptoms is poorly understood. Consequently, despite our strict inclusion criteria, it is unclear whether all symptoms in our study population are attributable to Lyme disease. Nevertheless, this population represents the patients who are actually encountered in clinical practice and who do suffer from low quality of life, have high healthcare consumption and productivity losses. Therefore, research regarding the effectiveness, costs and cost-effectiveness of treatment regimens in this patient group is essential for clinical practice.

Costs of potential antibiotic resistance among both the patients’ intestinal flora and the environment were not included in our evaluation. If these costs could have been taken into account, the longer-term treatment regimens likely would have been less favorable in terms of costs and cost-effectiveness compared to the shorter-term treatment. Antibiotic resistance is a growing global threat, which has been estimated to cause 23,000 deaths and $55 billion of healthcare costs and productivity losses each year in the United States. In Europe, these numbers were estimated to be 25,000 deaths and €1.5 billion yearly. Since antibiotic resistance is directly related to volumes of antibiotic treatment and since resistance of both first-line and last-resort antibiotics is increasing rapidly, ineffective and even potentially harmful treatment with antibiotics should be prevented.

**CONCLUSIONS**

From a societal perspective, the longer-term treatments of ceftriaxone combined with doxycycline or with clarithromycin and hydroxychloroquine were as costly, effective and cost-effective as shorter-term treatment with ceftriaxone only in patients with persistent symptoms attributed to Lyme disease after one year of follow-up. Taking into account the growing concern to antibiotic resistance because of unnecessary use, the shorter-term provision of antibiotics should be preferred.
REFERENCES


Effect prolonged antibiotic treatment on cognitive performance in patients with Lyme borreliosis

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Roy P.C. Kessels
Bart Jan Kullberg

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ABSTRACT

Objective: To investigate whether longer-term antibiotic treatment improves cognitive performance in patients with persistent symptoms attributed to Lyme borreliosis.

Methods: Data were collected during the Persistent Lyme Empiric Antibiotic Study Europe (PLEASE) trial, a randomized, placebo-controlled study. Study participants passed performance-validity testing (measure for detecting suboptimal effort) and had persistent symptoms attributed to Lyme borreliosis. All patients received a 2-week open-label regimen of intravenous ceftriaxone before the 12-week blinded oral regimen (doxycycline, clarithromycin/hydroxychloroquine, or placebo). Cognitive performance was assessed at baseline and after 14, 26, and 40 weeks with neuropsychological tests covering the cognitive domains of episodic memory, attention/working memory, verbal fluency, speed of information processing, and executive function.

Results: Baseline characteristics of patients enrolled (n = 239) were comparable in all treatment groups. After 14 weeks, performance on none of the cognitive domains differed significantly between the treatment arms (∆p = 0.49 - 0.82). At follow-up, no additional treatment effect (∆p = 0.35 - 0.98) or difference between groups was found at any time-point (p = 0.37 - 0.93). Patients performed significantly better in several cognitive domains at week 14, 26 and 40 compared to baseline, but this was not specific to a treatment group.

Conclusions: A 2-week treatment with ceftriaxone followed by a 12-week regimen of doxycycline or clarithromycin/hydroxychloroquine did not lead to better cognitive performance compared to a 2-week regimen of ceftriaxone in patients with Lyme disease-attributed persistent symptoms.

Classification of Evidence: This study provides Class II evidence that longer-term antibiotics in patients with borreliosis-attributed persistent symptoms does not increase cognitive performance compared to shorter-term antibiotics.
INTRODUCTION

Many patients who experience persistent symptoms that are attributed to Lyme borreliosis complain of cognitive problems such as memory loss, word-finding difficulties, and concentration problems. However, previous studies have failed to show significant correlations between subjective memory complaints and objective test performances in patients with Lyme borreliosis and other patients. This makes assessing neurocognitive function with objective neuropsychological tests important.

Several small studies have investigated the neurocognitive performance of patients with Lyme disease compared to healthy participants. Most found a worse performance in the patient group. Deficits observed in patients with persistent symptoms attributed to Lyme disease are best typified as a combination of reduced processing speed and memory problems.

To date, it is unknown whether the cognitive problems reported by patients with persistent Lyme disease-attributed symptoms are due to an insufficiently treated low-grade Borrelia burgdorferi infection, remnants of past infection, or incorrect attribution to Lyme borreliosis. Although most guidelines recommend antimicrobial therapy for a maximum of 2 to 4 weeks, others recommend longer-term antibiotic treatment.

Previous studies have not been conclusive in proving effects of longer-term antibiotic therapy on cognition. Furthermore, the trials performed were small (n = 129 and n = 37). The present study, the largest to date, was performed to evaluate the effect of prolonged antimicrobial treatment compared to shorter-term treatment on neurocognitive function in patients with symptoms attributed to Lyme borreliosis.

METHODS

STUDY DESIGN AND PARTICIPANTS

The data for this neurocognitive study were collected as secondary outcomes of the Persistent Lyme Empiric Antibiotic Study Europe (PLEASE), a multicenter, placebo-controlled, double-blind randomized clinical trial that was performed in the Netherlands at 2 locations (Sint Maartenskliniek and Radboud University Medical Center). From October 2010 through June
2013, patients were enrolled into this trial. The study design and protocol, inclusion and exclusion criteria,\textsuperscript{21} and main outcomes were previously published.\textsuperscript{22} Patients with ongoing symptoms such as musculoskeletal pain, neuralgia, sensory disturbances, or cognitive complaints were included if they also had \textit{B. burgdorferi} immunoglobulin (Ig)G or IgM antibodies or if the complaints were temporally linked to an erythema migrans or otherwise proven symptomatic Lyme borreliosis.

**STANDARD PROTOCOL APPROVALS, REGISTRATIONS, AND PATIENT CONSENTS**

The local ethics committee has approved the PLEASE protocol (CMO region Arnhem–Nijmegen, 2009/187, NL27344.091.09). All participants provided written informed consent. The trial was registered with ClinicalTrials.gov (NCT01207739).

**RANDOMIZATION AND MASKING**

Computerized randomization distributed patients into 3 groups in a 1:1:1 ratio. The randomization was balanced by minimization for duration of symptoms (<1 or ≥1 year), age (<40 or ≥40 years), sex, and baseline RAND-36 Health Status Inventory (RAND SF-36) Global Health Composite score.\textsuperscript{23} An independent web manager entered the randomization list, consisting of consecutive medication numbers, into a secured web-based database. None of the participants or personnel involved in the trial (apart from the web manager and study pharmacist) were aware of the assignments to study groups.

**CLASSIFICATION OF EVIDENCE**

The primary research question is whether longer-term antibiotic treatment with 2 weeks of ceftriaxone followed by 12 weeks of doxycycline or clarithromycin/hydroxychloroquine improves cognitive performance in patients with persistent symptoms attributed to Lyme borreliosis compared to shorter-term antibiotic treatment with 2 weeks of ceftriaxone. This trial provides Class II evidence that longer-term treatment does not lead to additional improvement.
INTERVENTION

All patients were treated with open-label intravenous ceftriaxone daily for 2 weeks. After completion, patients started on a blinded and randomized 12-weeks’ oral regimen of doxycycline, clarithromycin/hydroxychloroquine, or placebo. The study drugs and placebo had an identical appearance. More details on the intervention have been provided in the study protocol of the PLEASE trial. 21

PROCEDURES

Cognitive performance was assessed at baseline, after end of treatment (EOT) at 14 weeks, at 26 weeks, and at 40 weeks with an extensive neuropsychological test battery covering the 5 major cognitive domains: episodic memory, attention/working memory, fluency, speed of information processing and executive function. We measured episodic memory with the Rey Auditory Verbal Learning Test, attention/working memory with the Digit Span Test, language with the Category Fluency Test, speed of information processing with the Trail Making Test (TMT) part A, the average speed of Cards I and II from the Stroop Color-Word Test, and the Symbol-Digit Substitution Test. We assessed executive function with the Trail Making Test Interference Score (Part B/Part A) as well as the Stroop Interference Score (Card III/average of Cards I and II). The raw test scores were standardized into z scores by use of the pooled mean of baseline scores of the entire study sample. The compound score for each cognitive domain was obtained by calculating the mean of the z scores for tests making up that domain. Higher scores represent better performance. Further details on the neuropsychological assessment have been published previously in a report on our protocol. 21 Furthermore, we administered the Amsterdam Short Term Memory Test at baseline to identify participants who displayed suboptimal effort affecting performance validity. This test only appears to be a difficult task; even patients with brain damage can perform well. 24 Poor performance on this task indicates suboptimal mental effort. The cutoff score for this performance validity test is 85 points (maximum score 90), with a sensitivity of 86% and a specificity of 87%. Because we aimed to obtain an optimal specificity (i.e., >90%), we included only patients scoring ≥ 83 points (with a specificity of 93%) in the analyses to exclude participants who displayed suboptimal effort. 24, 25
FIGURE 1. Flow chart
STATISTICAL ANALYSIS

In this study, we report secondary outcomes of the main trial, the PLEASE study. The analyses include only patients who were randomly assigned to a study group, received at least 1 dose of ceftriaxone (modified intention-to-treat population), and displayed sufficient performance validity at baseline (Amsterdam Short Term Memory Test score ≥83). For descriptive purposes, we also classified individuals at baseline as having a clinically impaired cognitive performance using Multivariate Normative Comparisons based on a large Dutch normative data set from the Advanced Neuropsychological Diagnostic Infrastructure (ANDI).

We compared the 3 study groups at week 14 (EOT) with analysis of covariance, including baseline domain score as covariate. Missing data at week 14 were imputed if they occurred in <5% of the cases with the mean of the treatment group at that assessment moment. We performed linear mixed models to estimate the duration of the potential intervention effect, including all 3 post-treatment assessments (14, 26, and 40 weeks). All models contained the baseline value of the dependent variable, time, study group treatment, and time-by-treatment interaction.

The alpha level was set at 0.05 (2-tailed), and 95% confidence intervals are reported when appropriate. For pairwise comparisons of the 5 domains among the 3 study groups at different endpoints, Bonferroni correction was used (by adjusting alpha to 0.01) to reduce the probability of family-wise (type I) error. Sensitivity analyses included all analyses without imputation. SPSS software version 22, was used to perform the statistical analyses.

DATA AVAILABILITY

Anonymized data, related documents such as study protocol, and statistical analysis will be shared by request from any qualified investigator for 5 years after the date of publication.
Table 1. Baseline characteristics*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Ceftriaxone + doxycycline (n = 72)</th>
<th>Ceftriaxone + clarithromycin/hydroxychloroquine (n = 86)</th>
<th>Ceftriaxone + placebo (n = 81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex, no. (%)</td>
<td>33 (46)</td>
<td>37 (43)</td>
<td>39 (48)</td>
</tr>
<tr>
<td>Age, mean (± SD), y</td>
<td>48.3 (12.6)</td>
<td>47.5 (13.0)</td>
<td>50.3 (9.9)</td>
</tr>
<tr>
<td>White, no. (%)</td>
<td>70 (97)</td>
<td>86 (100)</td>
<td>81 (100)</td>
</tr>
<tr>
<td>Current symptoms, no. (%)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>67 (93)</td>
<td>77 (90)</td>
<td>72 (89)</td>
</tr>
<tr>
<td>Musculoskeletal pain</td>
<td>61 (85)</td>
<td>69 (80)</td>
<td>63 (78)</td>
</tr>
<tr>
<td>Sensory disturbances</td>
<td>50 (69)</td>
<td>67 (78)</td>
<td>65 (81)</td>
</tr>
<tr>
<td>Neuralgia</td>
<td>6 (8)</td>
<td>12 (14)</td>
<td>14 (17)</td>
</tr>
<tr>
<td>Neurocognitive symptoms</td>
<td>63 (88)</td>
<td>72 (84)</td>
<td>72 (89)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>70 (97)</td>
<td>82 (95)</td>
<td>76 (94)</td>
</tr>
<tr>
<td>Duration of symptoms, median (IQR), y</td>
<td>2.7 (1.3 – 7.6)</td>
<td>2.8 (1.4 – 5.5)</td>
<td>2.3 (0.9 – 6.2)</td>
</tr>
<tr>
<td>History of Lyme disease, no. (%)††</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tick bite</td>
<td>39 (55)</td>
<td>43 (51)</td>
<td>48 (60)</td>
</tr>
<tr>
<td>Erythema migrans†</td>
<td>21 (29)</td>
<td>22 (26)</td>
<td>24 (30)</td>
</tr>
<tr>
<td>Acrodermatitis chronica atrophicans§</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Borrelia meningoradiculitis **</td>
<td>1 (1)</td>
<td>8 (9)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Previous use of antimicrobial treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, no. (%)</td>
<td>64 (89)</td>
<td>77 (90)</td>
<td>73 (90)</td>
</tr>
<tr>
<td>Duration, median (IQR), d</td>
<td>40 (28 – 56)</td>
<td>30 (21 – 44)</td>
<td>31 (28 – 55)</td>
</tr>
<tr>
<td>Education level, no. (%)††</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (≤8 y of education)</td>
<td>1 (1.4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Average (9-11 y of education)</td>
<td>39 (54.9)</td>
<td>40 (46.5)</td>
<td>34 (42.5)</td>
</tr>
<tr>
<td>High (≥12 y of education)</td>
<td>31 (43.7)</td>
<td>46 (53.5)</td>
<td>46 (57.5)</td>
</tr>
<tr>
<td>Employment, no. (%)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working</td>
<td>37 (51.4)</td>
<td>58 (67.4)</td>
<td>59 (73.8)§§</td>
</tr>
<tr>
<td>Student</td>
<td>3 (4.2)</td>
<td>5 (5.8)</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>Disabled or on sick leave</td>
<td>29 (40.3)</td>
<td>28 (32.6)</td>
<td>24 (29.6)</td>
</tr>
<tr>
<td>Retired</td>
<td>9 (12.5)</td>
<td>6 (7.0)</td>
<td>7 (9.2)</td>
</tr>
<tr>
<td>Cognitive domain compound score, mean (95% CI)‡‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Episodic memory</td>
<td>-0.08 (-0.29 – 0.12)</td>
<td>0.06 (-0.12 – 0.24)</td>
<td>0.19 (0.02 – 0.37)</td>
</tr>
<tr>
<td>Attention/working memory</td>
<td>-0.11 (-0.35 – 0.13)</td>
<td>0.26 (0.06 – 0.46)</td>
<td>0.06 (-0.17 – 0.29)</td>
</tr>
</tbody>
</table>
Table 1. (Continued)

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal fluency</td>
<td>-0.09 (-0.31 – 0.13)</td>
<td>-0.01 (-0.22 – 0.20)</td>
<td>0.18 (-0.06 – 0.41)</td>
</tr>
<tr>
<td>Speed of information processing</td>
<td>0.00 (-0.20 – 0.19)</td>
<td>0.12 (-0.04 – 0.28)</td>
<td>0.10 (-0.08 – 0.27)</td>
</tr>
<tr>
<td>Executive function</td>
<td>-0.02 (-0.21 – 0.17)</td>
<td>0.04 (-0.14 – 0.23)</td>
<td>0.11 (-0.06 – 0.27)</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval; IQR = interquartile range.
* Between-group differences were analyzed with χ² tests for proportions, analysis of variance for continuous variables, and Fisher exact test for small numbers. Kruskal-Wallis tests were used for ordinal and not normally distributed data.
† Categories are not mutually exclusive.
‡ Temporally related: physician-confirmed diagnosis, maximum 4 months before onset of symptoms.
§ Temporally related: biopsy or physician-confirmed diagnosis, maximum 4 months before onset of symptoms.
** Temporally related: diagnosis by intrathecal Borrelia immunoglobulin G synthesis, maximum 4 months before onset of symptoms.
†† Education was assessed in accordance with the Dutch education system.30
‡‡ The z scores were computed from the pooled mean of baseline scores of the entire study sample. For each cognitive domain, a compound score was derived by computing the mean of the z scores for tests making up that domain. Higher scores represent better performance.
§§ p < 0.05.

RESULTS

Of the 281 patients randomized, 85% (n = 239) displayed sufficient performance validity on the cognitive tests at baseline (figure 1). No baseline differences were found between the 3 treatment groups, including baseline neuropsychological performance (table 1), apart from the percentage of patients with a job, which significantly differed between groups. At baseline, 7 of 239 patients were classified as having a clinically impaired cognitive performance compared to Dutch normative data.

The neuropsychological performance (i.e., the mean z score per domain) at EOT (14 weeks), corrected for baseline performance and sex, did not significantly differ between treatment groups for any of the domains, with p values ranging from 0.49 to 0.82 (table 2).

Figure 2 shows the mean performance per group for each neuropsychological domain over time. The differences between the various time points compared to baseline are depicted in table 3. The performance on 2 domains, episodic memory and speed of information, significantly improved between baseline and EOT in all randomization groups. Similarly, at 26 and 40 weeks, several domains showed higher scores compared to baseline.

However, no additional long-term treatment effects were seen using mixed-model analyses (the difference between the treatment arms did not change over time) for any of the domains; p values ranged from 0.35 to 0.98 for the time-by-treatment interaction. No
significant difference was found between the 3 treatment groups at any time point during follow-up in neuropsychological performance either ($p$ values ranging from 0.37 to 0.93). All sensitivity analyses yielded results similar to those of the main analyses. Several post hoc analyses were also done. Subset analyses with patients who had symptoms for <1 year ($n = 46$) did not show a significant difference between treatment groups. Excluding patients who did not report subjective cognitive complaints at baseline ($n = 32$) did not yield different results, neither did post hoc analyses on the subgroup of patients with severe subjective symptoms as measured by the Cognitive Failures Questionnaire (CFQ). With a cutoff value for the CFQ set at 44, 111 patients were considered to have severe neurocognitive symptoms. Finally, subgroup analyses including only patients who had a high burden of symptoms (i.e., those who were on sick leave or disability support, $n = 81$) also did not show a significant difference between placebo and antimicrobial treatment groups. Using analysis of covariance, with sick leave/disability and baseline cognitive function as covariates, we found no significant difference between treatment groups.

**Table 2. Neuropsychological performance at EOT (14 weeks)**

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Ceftriaxone + doxycycline ($n = 72$)</th>
<th>Ceftriaxone + clarithromycin/hydroxychloroquine ($n = 86$)</th>
<th>Ceftriaxone + placebo ($n = 81$)</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Episodic memory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean z score (95% CI)</td>
<td>0.19 (0.03 - 0.35)</td>
<td>0.27 (0.13 - 0.41)</td>
<td>0.27 (0.12 - 0.42)</td>
<td>0.70</td>
</tr>
<tr>
<td>difference with placebo (95% CI)</td>
<td>-0.08 (-0.34 - 0.18)</td>
<td>0.00 (-0.25 - 0.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Attention / working memory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean z score (95% CI)</td>
<td>0.21 (0.05 - 0.37)</td>
<td>0.16 (0.01 - 0.30)</td>
<td>0.25 (0.10 - 0.40)</td>
<td>0.65</td>
</tr>
<tr>
<td>difference with placebo (95% CI)</td>
<td>-0.04 (-0.30 - 0.22)</td>
<td>-0.10 (-0.35 - 0.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Verbal fluency</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean z score (95% CI)</td>
<td>0.18 (0.02 - 0.35)</td>
<td>0.24 (0.09 - 0.39)</td>
<td>0.13 (-0.03 - 0.28)</td>
<td>0.60</td>
</tr>
<tr>
<td>difference with placebo (95% CI)</td>
<td>0.06 (-0.22 - 0.34)</td>
<td>0.11 (-0.16 - 0.38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Speed of information processing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean z score (95% CI)</td>
<td>0.25 (0.15 - 0.36)</td>
<td>0.30 (0.21 - 0.39)</td>
<td>0.34 (0.24 - 0.44)</td>
<td>0.49</td>
</tr>
<tr>
<td>difference with placebo (95% CI)</td>
<td>-0.09 (-0.26 - 0.09)</td>
<td>-0.04 (-0.20 - 0.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Executive function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean z score (95% CI)</td>
<td>0.14 (-0.01 - 0.28)</td>
<td>0.13 (0.00 - 0.27)</td>
<td>0.19 (0.05 - 0.32)</td>
<td>0.82</td>
</tr>
<tr>
<td>difference with placebo (95% CI)</td>
<td>-0.05 (-0.30 - 0.19)</td>
<td>-0.05 (-0.29 - 0.18)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** CI = confidence interval; EOT = end of treatment.

* Between-group differences in characteristics were analyzed with analysis of covariance, adjusted for baseline domain score.
FIGURE 2. Mean z score (95% confidence interval) per treatment group per neuropsychological domain at all study visits.
Table 3. Treatment effect at different endpoints (14, 26, and 40 weeks compared to baseline)

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Week 14 vs baseline</th>
<th>Week 26 vs baseline</th>
<th>Week 40 vs baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>Difference</td>
<td>Difference</td>
</tr>
<tr>
<td></td>
<td>in mean z score (SEM)</td>
<td>in mean z score (SEM)</td>
<td>in mean z score (SEM)</td>
</tr>
<tr>
<td></td>
<td>P value*</td>
<td>P value*</td>
<td>P value*</td>
</tr>
<tr>
<td>Episodic memory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone + doxycycline</td>
<td>0.16 (0.08)</td>
<td>0.056</td>
<td>0.26 (0.07)</td>
</tr>
<tr>
<td>Ceftriaxone + clarithromycin</td>
<td>0.22 (0.08)</td>
<td>&lt;0.01</td>
<td>0.27 (0.07)</td>
</tr>
<tr>
<td>Ceftriaxone + placebo</td>
<td>0.19 (0.07)</td>
<td>&lt;0.01</td>
<td>0.14 (0.08)</td>
</tr>
<tr>
<td>Attention/working memory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone + doxycycline</td>
<td>0.18 (0.08)</td>
<td>0.031</td>
<td>0.26 (0.07)</td>
</tr>
<tr>
<td>Ceftriaxone + clarithromycin</td>
<td>0.04 (0.08)</td>
<td>0.589</td>
<td>0.11 (0.07)</td>
</tr>
<tr>
<td>Ceftriaxone + placebo</td>
<td>0.19 (0.07)</td>
<td>0.012</td>
<td>0.29 (0.09)</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone + doxycycline</td>
<td>0.19 (0.09)</td>
<td>0.033</td>
<td>0.32 (0.08)</td>
</tr>
<tr>
<td>Ceftriaxone + clarithromycin</td>
<td>0.20 (0.09)</td>
<td>0.025</td>
<td>0.39 (0.09)</td>
</tr>
<tr>
<td>Ceftriaxone + placebo</td>
<td>0.09 (0.08)</td>
<td>0.245</td>
<td>0.12 (0.10)</td>
</tr>
<tr>
<td>Speed of information processing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone + doxycycline</td>
<td>0.19 (0.05)</td>
<td>&lt;0.01</td>
<td>0.33 (0.05)</td>
</tr>
<tr>
<td>Ceftriaxone + clarithromycin</td>
<td>0.22 (0.05)</td>
<td>&lt;0.01</td>
<td>0.41 (0.06)</td>
</tr>
<tr>
<td>Ceftriaxone + placebo</td>
<td>0.26 (0.05)</td>
<td>&lt;0.01</td>
<td>0.40 (0.06)</td>
</tr>
<tr>
<td>Executive function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone + doxycycline</td>
<td>0.11 (0.07)</td>
<td>0.1290</td>
<td>0.22 (0.08)</td>
</tr>
<tr>
<td>Ceftriaxone + clarithromycin</td>
<td>0.10 (0.08)</td>
<td>0.1884</td>
<td>0.20 (0.10)</td>
</tr>
<tr>
<td>Ceftriaxone + placebo</td>
<td>0.11 (0.09)</td>
<td>0.2013</td>
<td>0.10 (0.08)</td>
</tr>
</tbody>
</table>

*Bonferroni correction was applied, i.e. alpha was adjusted to 0.01.
DISCUSSION

This study showed that prolonged antibiotic treatment for 3 months in patients with persistent Lyme borreliosis-attributed symptoms does not have an additional beneficial effect on cognitive performance compared to short-term treatment.

Previous case series have suggested a significant cognitive improvement on most domains after antibiotic treatment.\(^{18,19}\) Two randomized controlled treatment trials have also demonstrated significant improvement of objective test scores after treatment compared to baseline performance.\(^{5,17}\) However, no significant differences were found between those receiving antibiotics and placebo in 1 trial,\(^{5}\) and the other trial did not show sustainable effects of antibiotic treatment on cognition.\(^{17}\) In our trial, mixed-model analyses showed no difference over time. Cognitive improvements were only found at weeks 14, 26 and 40 only when the separate domains were directly compared with baseline, and changes over time were at most in the small to moderate range. Because an improvement was seen in all treatment groups, including the placebo control group, the observed changes appear to be neither clinically relevant nor treatment specific. The global difference found over time may be the result of a placebo effect, nonspecific practice effects, spontaneous improvement over time, or a combination of these.

The present study is the largest trial performed to date. It was specifically designed prospectively to study treatment outcomes, including cognitive performance, using a strictly controlled design.\(^{21,22}\) In addition, our study is the first to take suboptimal cognitive effort into account in the neuropsychological assessment by selecting only patients who displayed sufficient performance validity. Kaplan et al.\(^{5}\) have investigated the personality traits of participants and investigated symptom validity to some extent by examining the patients’ ability to present a false impression using the Minnesota Multiphasic Personality Inventory-2. However, that does not compare to our way of taking suboptimal cognitive effort explicitly into account using performance validity testing.

A limitation in our study may relate to missing values. To reduce the influence of missing values, mixed-model analyses were performed. In these analyses, no significant differences between groups on any of the domains were observed either.

While a ceiling effect may be considered, because only a few patients were overall cognitively impaired at baseline, none of the raw scores were at or near ceiling for any
of the tests at various endpoints. The mean performances per test were typically in the
midrange between the minimally and maximally possible scores, leaving sufficient room
for improvement.

The fact that we did not include only patients with subjective cognitive complaints could
be seen as another limitation. However, our patient population is representative for the real-
life population of patients with Lyme borreliosis, improving the external validity. Moreover,
only 32 of 280 patients did not report subjective cognitive complaints at baseline. Post
hoc analyses excluding those 32 patients did not yield different results; i.e., there was no
significant difference between groups at EOT.

Finally, because the study was not specifically powered for detecting neuropsychological
test outcomes, the results must be seen as preliminary.

Future studies on treatment of cognitive function in individuals with Lyme borreliosis
may specifically focus on the small group of patients with objectively impaired cognitive
performance.

Our study suggests that cognitive performance as assessed by validated tests does not
improve by longer antibiotic treatment compared to shorter-term treatment in patients with
persistent symptoms attributed to Lyme borreliosis.

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REFERENCES


Cognitive impairments in patients with persistent symptoms attributed to Lyme disease

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ABSTRACT

Background: Persistent symptoms attributed to Lyme borreliosis often include self-reported cognitive impairment. However, it remains unclear whether these symptoms can be substantiated by objective cognitive testing.

Methods: For this observational study, cognitive performance was assessed in 280 adults with persistent symptoms attributed to Lyme borreliosis (as part of baseline data collected for the Dutch PLEASE study). Cognitive testing covered the five major domains: episodic memory, working memory/attention, verbal fluency, information-processing speed and executive function. Patients’ profiles of test scores were compared to a large age-, education- and sex-adjusted normative sample using multivariate normative comparison. Performance validity was assessed to detect suboptimal effort, and questionnaires were administered to measure self-reported cognitive complaints, fatigue, anxiety, depressive symptoms and several other psychological factors.

Results: Of 280 patients, one was excluded as the test battery could not be completed. Of the remaining 279 patients, 239 (85.4%) displayed sufficient performance validity. Patients with insufficient performance validity felt significantly more helpless and physically fatigued, and less orientated. Furthermore, they had a lower education level and less often paid work. Of the total study cohort 5.7% (n = 16) performed in the impaired range. Among the 239 patients who displayed sufficient performance validity, 2.9% (n = 7) were classified as cognitively impaired. No association between subjective cognitive symptoms and objective impairment was found.

Conclusions: Only a small percentage of patients with borreliosis-attributed persistent symptoms have objective cognitive impairment. Performance validity should be taken into account in neuropsychological examinations of these patients. Self-report questionnaires are insufficiently valid to diagnose cognitive impairment.
BACKGROUND

Patients with persistent symptoms attributed to Lyme borreliosis often report a variety of cognitive symptoms. However, subjective cognitive symptoms are not always due to underlying cognitive impairments. Previously, subjective ratings of memory capabilities and objective memory performance were only weakly correlated in patients with post-treatment Lyme disease \(^1\). Most previous studies that compared cognitive performance of Lyme patients to healthy controls found a worse performance in Lyme patients at group level \(^2\)-\(^{12}\). The most affected cognitive domain was episodic memory. Findings concerning the domains verbal fluency and processing speed were less consistent. However, most studies were relatively small (n<80), originated from the US, and used diverse inclusion criteria and methods. In the 4 larger US studies, mild to no cognitive abnormalities were identified \(^1\), \(^2\), \(^{12}\), \(^{13}\). As both the *Borrelia* species and the clinical presentation of Lyme disease in Europe and the US differ \(^14\), cognitive function in European Lyme patients requires separate assessment. Furthermore, most previous studies have not taken performance validity into account. This is crucial, as a suboptimal performance results in poor tests scores not reflecting an individual’s actual cognitive status. Very recently, the study by Touradji et al. in a group of US patients with post-treatment Lyme disease showed that 24% of the sample displayed suboptimal effort on measures of performance validity \(^{12}\). Hence, suboptimal performance affects the validity and reliability of neuropsychological outcomes, resulting in false positive results (i.e., patients incorrectly labelled as having a cognitive impairment) \(^{15}\). This stresses the need to take performance validity testing into account when cognitively assessing patients with persistent symptoms attributed to Lyme disease.

The aim of the present study was to objectively assess cognitive performance using sensitive tests in a large cohort of patients with persistent symptoms attributed to Lyme borreliosis, while taking performance validity into account, and to compare cognitive performance outcomes with subjective symptoms.
METHODS

The current study uses baseline data collected between 2010-2013 as part of the Persistent Lyme Empiric Antibiotic Study Europe (PLEASE). Previously, we reported the primary and secondary outcome measures of this multicenter, placebo-controlled, double-blind randomized controlled trial from the Netherlands (ClinicalTrials.gov NCT01207739)\textsuperscript{16,17}. The local Institutional Review Board approved the PLEASE protocol, and informed consent was obtained from each participant. Here we provide a detailed report on the baseline cognitive and self-report questionnaire data. The study population comprises adult patients (n = 280) referred with persistent symptoms attributed to Lyme borreliosis, preceded by confirmed symptomatic Lyme disease or accompanied by positive \textit{B. burgdorferi} IgG or IgM antibodies, as confirmed by means of immunoblot assay. Patients were not required to have received antibiotic treatment before study entry. Major symptoms included musculoskeletal pain, cognitive disturbances and/or fatigue. Details about inclusion and exclusion criteria have been published previously\textsuperscript{16}.

OUTCOMES

Cognitive performance was assessed using an extensive neuropsychological test battery covering five major cognitive domains: episodic memory, working memory/attention, verbal fluency, information-processing speed and executive function. Episodic memory was assessed using the Rey Auditory Verbal Learning Test (RAVLT), working memory/attention with the Digit Span, verbal fluency with the Category Fluency Test (animal/profession naming), and information-processing speed with the Trail Making Test Part A and the mean response time of cards I and II from the Stroop Color-Word Test, and the Symbol-Digit Substitution Test. Executive function was measured using the Interference Score of the Trail Making Test (Part B/Part A) and the Stroop Interference Score (card III/mean of cards I and II). Assessment details have been published previously\textsuperscript{17}.

To identify participants with insufficient performance validity, the Amsterdam Short Term Memory test (ASTM) was administered\textsuperscript{18}. A poor performance on this task indicates suboptimal mental effort. The recommended cut-off score is 85 (maximum score = 90), with 86\% sensitivity and 87\% specificity\textsuperscript{19}. However, since our goal was to prioritize optimal specificity (>90\%), adopting a conservative approach that reduces the risk of false alarms...
on performance validity tests (i.e., incorrectly labelling a participant as someone displaying suboptimal effort), we used a cut-off score of <83 (which has a specificity of 95% and a sensitivity of 76%).

Subjective measurement of cognitive function was assessed with the Cognitive Failures Questionnaire (CFQ) 20, fatigue by the Checklist Individual Strength (CIS) 16, anxiety and depressive symptoms by the Hospital Anxiety and Depression Scale (HADS) 21, self-efficacy by a modified version of the Arthritis Self-Efficacy Scale (i.e., ‘pain’ replaced by ‘physical symptoms’) 22, illness cognitions by the Illness Cognition Questionnaire (ICQ) 23, worrying by the Penn-State Worry Questionnaire (PSWQ) 24, neuroticism and extraversion by the Eysenck Personality Questionnaire (EPQ) 25, and fear of body sensations by the Body Sensations Questionnaire (BSQ) 26.

**STATISTICAL ANALYSIS**

First, we investigated which demographic/psychological factors were associated with poor performance validity. For patients with sufficient performance validity, we determined whether their cognitive performance was impaired by comparing individual test performances to an extensive normative sample (n = 26,939) from the Advanced Neuropsychological Diagnostics Infrastructure (ANDI) 27. We performed a multivariate normative comparison (MNC) on each patient’s neuropsychological test profile, applying corrections for age, sex and education level. The MNC provides an individual classification based on the profile of tests as either ‘cognitively impaired’ or ‘cognitively unimpaired’ 27.

We explored performance on individual cognitive domains, averaging age-, sex- and education-adjusted z-scores per domain. A domain was classified as ‘impaired’ if z<-1.5 (i.e., more than 1.5 SD below the normative mean). The relation between objective cognitive functioning and subjective complaints was analyzed with Pearson correlation coefficients.

Alpha was set at 0.05 throughout (two-tailed), and 95% confidence intervals are reported when appropriate. Benjamini-Hochberg correction was used to reduce the false discovery rate for multiple comparisons, accepting a false discovery rate of 0.10.
RESULTS

Of the 280 patients included, one was unable to perform several neuropsychological tests due to visual impairment unrelated to Lyme disease. Of the 279 patients fully examined, 239 (85.4%) displayed sufficient performance validity.

Table 1 shows patient characteristics stratified by performance validity status. Patients with insufficient performance validity had significantly lower education levels and less often paid work. They also reported significantly more feelings of helplessness (ICQ subscale helplessness, F(1,275) = 9.77), experienced more physical fatigue (CIS Activity subscale, F(1,276) = 6.79), and reported more problems in daily orientation (CFQ Orientation subscale, F(1,276) = 8.40).

Table 1. Demographic and psychosocial factors stratified by performance validitya

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Good performance validity (n=239)</th>
<th>Poor performance validity (n=40)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women, no. (%)</td>
<td>109 (45.6)</td>
<td>19 (47.5)</td>
<td>0.82</td>
</tr>
<tr>
<td>Education level, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (≤8 years)</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Average (9-11 years)</td>
<td>125 (52.7)</td>
<td>28 (71.8)</td>
<td></td>
</tr>
<tr>
<td>High (≥12 years)</td>
<td>111 (46.8)</td>
<td>11 (28.2)</td>
<td></td>
</tr>
<tr>
<td>Paid work, no. (%)</td>
<td>154 (64.7)</td>
<td>17 (43.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>Age, mean (± SD), years</td>
<td>48.7 (11.9)</td>
<td>49.0 (11.9)</td>
<td>0.87</td>
</tr>
<tr>
<td>Duration of symptoms, median (IQR), years</td>
<td>2.7 (1.3 – 6.3)</td>
<td>1.8 (0.7 – 5.7)</td>
<td>0.13</td>
</tr>
<tr>
<td>Previous antibiotic treatment for Lyme disease, no.</td>
<td>213 (89.1)</td>
<td>33 (82.5)</td>
<td>0.23</td>
</tr>
<tr>
<td>Delay symptom onset and treatment, median (IQR), weeks</td>
<td>22.5 (3.0 – 103.5)</td>
<td>15.5 (2.0 – 69.0)</td>
<td>0.41</td>
</tr>
<tr>
<td>History of meningoradiculitis, no. (%)c (neuroborreliosis)</td>
<td>18 (7.5)</td>
<td>3 (7.7)</td>
<td>0.97</td>
</tr>
<tr>
<td>CFQ, mean (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orientation</td>
<td>3.92 (3.65 - 4.18)</td>
<td>4.98 (4.20 - 5.75)</td>
<td>0.004a</td>
</tr>
<tr>
<td>Distractibility</td>
<td>11.29 (10.71 - 11.87)</td>
<td>12.98 (11.58 - 14.37)</td>
<td>0.03</td>
</tr>
<tr>
<td>Blunders</td>
<td>7.04 (6.70 - 7.38)</td>
<td>7.95 (7.15 - 8.75)</td>
<td>0.05</td>
</tr>
<tr>
<td>Memory</td>
<td>7.32 (7.05 - 7.59)</td>
<td>7.03 (6.28 - 7.77)</td>
<td>0.43</td>
</tr>
<tr>
<td>Total</td>
<td>43.23 (41.52 - 44.94)</td>
<td>47.73 (43.24 - 52.21)</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Compared to the normative sample, 2.9% of patients (7/239) were cognitively impaired. For the separate domains, 2.1% were impaired on episodic memory (5/239), 5.4% on working memory/attention (13/239), 0.8% on verbal fluency (2/239), 2.1% on information-processing speed (5/239), and 0.4% on executive function (1/239). Table 2 shows the raw neuropsychological test scores as well as mean z-scores, and percentage of individuals with a cognitive decrement (z-score <1.0 SD below the age, sex and education-adjusted normative mean).
## Table 2. Neuropsychological test scores per domain and per test\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Good performance validity</th>
<th>Poor performance validity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=239)</td>
<td>(n=40)</td>
</tr>
<tr>
<td></td>
<td>mean raw score (SD)</td>
<td>mean z-score (SD)</td>
</tr>
<tr>
<td></td>
<td>mean z-score (SD)</td>
<td></td>
</tr>
<tr>
<td><strong>Episodic memory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAVLT (immediate recall, total of trials 1-5)</td>
<td>-0.12 (0.71)</td>
<td>-0.40 (0.81)</td>
</tr>
<tr>
<td>RAVLT (delayed recall)</td>
<td>9.4 (2.8)</td>
<td>-0.11 (0.80)</td>
</tr>
<tr>
<td><strong>Working memory/attention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Span</td>
<td>15.4 (3.3)</td>
<td>0.11 (1.01)</td>
</tr>
<tr>
<td><strong>Language</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category Fluency (animals)</td>
<td>25.4 (5.8)</td>
<td>-0.03 (0.79)</td>
</tr>
<tr>
<td>Category Fluency (professions)</td>
<td>19.1 (4.8)</td>
<td>0.07 (0.91)</td>
</tr>
<tr>
<td><strong>Information-processing speed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trail Making Test part A(^b)</td>
<td>30.5 (11.5)</td>
<td>0.19 (0.84)</td>
</tr>
<tr>
<td>Stroop Color-Word Test (Card I)(^b)</td>
<td>44.1 (8.7)</td>
<td>-0.10 (0.99)</td>
</tr>
<tr>
<td>Stroop Color-Word Test (Card II)(^b)</td>
<td>58.9 (12.5)</td>
<td>-0.23 (1.10)</td>
</tr>
<tr>
<td>Symbol-Digit Substitution Test</td>
<td>57.6 (11.3)</td>
<td>0.05 (0.99)</td>
</tr>
<tr>
<td><strong>Executive functions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trail Making Test interference score (Part B/Part A)(^b)</td>
<td>2.3 (0.7)</td>
<td>-0.13 (0.99)</td>
</tr>
<tr>
<td>Stroop interference score (Card III/average Card I and II)(^b)</td>
<td>1.8 (0.3)</td>
<td>0.78 (0.71)</td>
</tr>
</tbody>
</table>

**Abbreviations:** RAVLT = Rey Auditory Verbal Learning Test
\(^a\) mean standardized age, sex and education-adjusted normative z-scores(SD) are presented for the domains, mean raw scores(SD), as well as mean z-scores(SD), and percentage of subjects with a cognitive decrement (z-score <1.0 SD below the age, sex and education-adjusted normative mean) are presented for the separate tests. Higher scores represent better cognitive performance, unless otherwise indicated.
\(^b\) higher scores represent worse cognitive performance
No significant correlation between overall cognitive performance and subjective cognitive complaints (CFQ total score) was found in patients with sufficient performance validity ($r = 0.120, p = 0.064$). A significant correlation was found between objective performance and problems in orientation (CFQ subscale orientation; $r = 0.246$ ($p<0.001$). Performance and other CFQ subscales did not show any correlations ($p = 0.10-0.98$).

**DISCUSSION**

We have assessed neurocognitive function in the largest sample of patients with persistent symptoms attributed to Lyme disease so far, using sensitive tests and extensive normative data. Furthermore, this study was the first European study to take performance validity into account. In our study, 15% of patients displayed insufficient performance validity using a conservative cut-off that prioritizes specificity over sensitivity (i.e., reducing the chance of incorrectly labelling an individual as displaying poor performance validity), indicating that their neuropsychological test scores cannot be validly interpreted as they might not reflect the patients’ actual cognitive abilities. Of note, there remains a possibility that this group with insufficient performance validity contains legitimate poor performers. For example, they may have been too fatigued to perform sufficiently. Our percentage of insufficient performance is considerably lower than in the study of Touradji et al. who found that 24% of their sample of post-treatment Lyme disease patients showed suboptimal effort, which may have been due to our conservative approach in assessing suboptimal effort. These substantial proportions of patients displaying suboptimal effort, however, illustrate the need for performance validity testing when assessing cognition in patients with persistent neuropsychological symptoms. Patients who displayed insufficient effort reported more fatigue, memory-orientation difficulties, and more feelings of helplessness than the optimal performers. Additionally, these patients were more often without paid work and had lower education levels. It should also be noted that only 22.5% (9/40) of the individuals who displayed suboptimal effort would have been classified as ‘cognitively impaired’ based on the performance on all other neuropsychological tests. After exclusion of patients with poor performance validity, only 2.9% had impaired cognitive function. This rate is low compared to the high level of cognitive complaints reported by patients (on average...
about 1 SD above the normative mean on the CFQ)\textsuperscript{20}. The lack of correlation between objective and subjective cognitive functioning, was also reported in another large study in patients with Lyme-associated symptoms\textsuperscript{1}. This lack of correlation between objective cognitive performance and subjective cognitive complaints is not specific for Lyme, but has been demonstrated in other disorders as well (including HIV, dementia, and rheumatoid arthritis)\textsuperscript{28-30}. Subjective cognitive complaints are often associated with depressive symptoms\textsuperscript{29}. The percentage of 2.9% cognitively impaired patients is comparable to what is found in the normal population (i.e., by definition 2.3% of a normative sample performs worse than 2 SD below the normative mean). Similar to our results, the study by Kaplan et al.\textsuperscript{1} found only a small percentage of cognitively impaired individuals. That study also examined personality characteristics, albeit with a smaller sample size and a less extensive test battery than the present study. Another large study did not find any differences between patients and healthy controls in cognitive function either\textsuperscript{2}. However, a very recent large study, which did take performance validity into account, found a much higher percentage\textsuperscript{12}. Furthermore, two studies by Keilp et al. found distinctive cognitive difference between patients with symptoms attributed to Lyme disease and healthy controls\textsuperscript{6,7}. We can only speculate on an explanation for the difference in impairment for the patients with sufficient performance. Possibly, differences between \textit{Borrelia} species in the US and Europe may play a role. In addition, differences in recruitment bias across the various studies may also have played a role. For instance, in the paper by Touradji et al.\textsuperscript{12} it is stated that participants partially were self-referred, whereas our patients were all referred to the study centers by a primary care physician or medical specialty.

In addition to the low prevalence of cognitive impairments in our study, the pathogenesis of impaired cognition in relation to Lyme disease is still unclear, with scarce evidence for underlying central nervous system pathology\textsuperscript{31}.

A potential limitation of the present study is the absence of a contemporaneous control group of healthy individuals. However, we compared the individuals’ performances to a substantially larger normative sample, with specific adjustments for age, education and sex, than would have been ever possible with recruiting our own controls. Additionally, the fact that our study population was more heterogenous than previous studies could be seen as a limitation, and not all patients received previous treatment with antibiotics.
CONCLUSIONS

The present study, taking performance validity into account in a large, well-defined cohort of patients with persistent symptoms attributed to Lyme borreliosis, demonstrates that only a small percentage of patients can be classified as cognitively impaired. Furthermore, self-reported symptoms of cognitive problems are unrelated to performance on neuropsychological tests in patients with Lyme-associated symptoms.
REFERENCES


Cognitive impairments in patients with persistent symptoms attributed to Lyme disease
Expectancies as predictors of symptom improvement after antimicrobial therapy for persistent symptoms attributed to Lyme disease

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Bart Jan Kullberg
Andrea W.M. Evers

Submitted
ABSTRACT

Objectives: Expectancies about symptom improvement or deterioration are reliable predictors of symptom progression and treatment outcomes in a broad variety of (non-)pharmacological studies and treatments. The current study examined the role of expectancies in predicting primary outcomes of symptom improvement after antimicrobial therapy for persistent symptoms attributed to Lyme disease.

Design: Predictive study on data from a randomised placebo-controlled trial (PLEASE) comparing two longer-term antibiotic treatment arms to short-term antibiotic treatment.

Setting: A tertiary university hospital and a specialty hospital in the Netherlands.

Participants: At end-of-treatment (14 weeks after trial start) and follow-up (52 weeks), complete data were available of 231 and 170 (of an initial 280) patients with persistent symptoms temporally related to a history of erythema migrans or otherwise confirmed symptomatic Lyme disease, or accompanied by *B. burgdorferi* IgG or IgM antibodies, respectively.

Interventions: Two weeks of open-label intravenous ceftriaxone was followed by randomised 12-weeks of doxycycline, clarithromycin-hydroxychloroquine, or placebo.

Main outcome measures: Physical and mental health-related quality of life (HRQoL) were assessed before trial medication (pre-treatment functioning), at end-of-treatment, and at follow-up.

Results: Pre-treatment expectancies regarding symptom improvement were consistently associated with stronger physical and mental HRQoL improvements at both end-of-treatment and follow-up (95% CI-range total group: .09; .54, p<.01 to .27; .92, p<.001). Post-treatment expectancies regarding the treatment received, i.e. presuming to have received antibiotics vs. placebo, was also associated with more improvement at end-of-treatment, but not at
follow-up, on all outcome measures (95% CI-range 1.00;4.75, p = .003 to -7.34; -2.22, p<.001).

Conclusions: The present study adds to the PLEASE trial outcomes in showing how patients’ pre- and post-treatment expectancies regarding improvement of persistent Lyme-associated symptoms can explain part of the treatment outcome in terms of a more beneficial symptom course. Interventions altering expectancies of patients (e.g., communication about treatment success) thus offer potential new ways to improve patient outcomes.
INTRODUCTION

Large numbers of patients present with persistent symptoms attributed to infection with *Borrelia burgdorferi*.\(^1,2\) These patients mainly experience disabling symptoms of pain, fatigue, and neurological and cognitive disturbances. The most commonly provided current medical treatment consists of either shorter-term (2-4 weeks) or longer-term (≥ 3 months) antimicrobial therapy. Previous studies have indicated that both shorter- and longer-term antimicrobial therapies do not offer a sufficient cure for many patients with persistent symptoms.\(^3-6\) Thus, it is relevant to know which factors determine symptom progression and predict heterogeneity in treatment response, as this could offer potential new ways to improve patient outcomes.

Various demographic, disease-related, and individual characteristics, as well as pre-treatment functioning, have been related to treatment outcomes in previous studies in diverse populations, including patients with persistent symptoms attributed to Lyme disease. However, the results were inconsistent, limiting the clinical implications of these findings.\(^7-13\) More recently, particular interest has been devoted to the role of expectancies that patients have regarding their symptom progression and treatment outcomes. In many placebo-controlled trials, expectancies are reliable predictors of treatment outcomes in a broad variety of pharmacological and non-pharmacological treatments.\(^14-23\) Moreover, it has been shown that the drug that patients thought they received (active or placebo) was stronger related to outcome than the actual drug received.\(^24,25\) As increasingly acknowledged and starting to be applied in clinical populations,\(^26-34\) expectancies may thus strengthen or even (partly) determine the effects of these treatments.

The current study examines the role of pre- and post-treatment expectancies in comparison to other individual characteristics in predicting primary outcomes of symptom improvement after antimicrobial therapy for persistent symptoms attributed to Lyme disease. We hypothesized that, in addition to pre-treatment functioning, particularly pre-treatment expectancies regarding symptom improvement would be predictive of changes in physical and mental health-related quality of life (HRQoL) immediately after treatment and at the longer term.
METHODS

PARTICIPANTS AND PROCEDURE
The current study concerns a secondary analysis of the data collected as part of the Persistent Lyme Empiric Antibiotic Study Europe (PLEASE), a multicentre, placebo-controlled, double-blind randomised controlled trial conducted at two tertiary health centres in the Netherlands, the Radboud university medical center and the Sint Maartenskliniek. The PLEASE study aimed to examine whether longer-term antimicrobial therapy would lead to better patient outcomes than shorter-term therapy in patients with Lyme borreliosis-attributed persistent symptoms. The trial was registered at ClinicalTrials.gov (NCT01207739) and its design and main results have been reported in previous papers. In short, patients with persistent symptoms (e.g., pain, musculoskeletal symptoms, neuralgia, sensory disturbances, neuropsychological complaints, fatigue) that were either temporally related to a history of an erythema migrans (EM) or otherwise confirmed symptomatic Lyme disease, or accompanied by *B. burgdorferi* IgG or IgM antibodies were included into the trial. All participants received 2000 mg open-label intravenous ceftriaxone daily for two weeks (shorter-term treatment) before starting a blinded oral antibiotic regimen of 12 weeks (longer-term treatment), for which they were randomly allocated in a 1:1:1 ratio to one of three treatment arms: 1) 100 mg of doxycycline twice daily plus placebo twice daily; 2) 500 mg clarithromycin twice daily plus 200 mg hydroxychloroquine twice daily; or 3) two placebos twice daily. The data for the current study were derived from questionnaires assessed at study start, at 14 weeks (end-of-treatment; primary outcome assessment point) and 52 weeks (long-term follow-up) after study start. The study was ethically approved by the Medical Ethics Review Committee CMO Region Arnhem-Nijmegen and all participants gave written informed consent.

INSTRUMENTS

PRIMARY AND SECONDARY OUTCOME MEASURES
In line with the PLEASE trial, the same primary and secondary physical and mental health-related quality of life (HRQoL) outcome measures were examined in this study.
PHYSICAL HRQOL:

**Physical component summary score:** As primary outcome, the physical component summary score (PCS) of the RAND-36 Health Status Inventory (RAND SF-36) was assessed. This score is calculated as norm-based T-score from the four weighted physical RAND SF-36 subscales (physical functioning, role limitations due to physical health problems, pain, and general health perceptions); these T-scores range from 15 to 61 and have a mean of 50 and standard deviation of 10 in the general population, with higher scores indicating a better physical HRQoL.

**Fatigue severity:** Fatigue, which is a frequent symptom that is not measured within the PCS of the RAND SF-36, was assessed as a secondary outcome by means of the severity of fatigue subscale of the Checklist Individual Strength (CIS). This is an 8-item scale with a score range of 8 to 56, with a mean of 17 and a standard deviation of 10 in a healthy sample. Higher scores on this measure indicate more severe fatigue.

MENTAL HRQOL:

**Mental component summary score:** As another secondary outcome, the mental component summary score (MCS) of the RAND SF-36 was assessed, calculated as norm-based T-scores from the four weighted mental RAND SF-36 subscales (emotional well-being, role limitations due to emotional problems, social functioning, and energy). The T-scores on this measure range from 11 to 66, also with a mean of 50 and standard deviation of 10 in the general population, with higher scores indicating a better mental HRQoL.

PREDICTORS OF SYMPTOM IMPROVEMENT AFTER ANTIMICROBIAL THERAPY

Three categories of predictor variables were assessed: 1) demographic, disease-related, and study-related characteristics, 2) pre-treatment functioning, and 3) individual characteristics, including expectancies.

**DEMOCRATIC, DISEASE-RELATED, AND STUDY-RELATED CHARACTERISTICS:**

The following demographic characteristics were assessed pre-treatment: age, sex, marital status, education level, smoking, and paid labour. Disease-related factors assessed pre-treatment were duration of Lyme-related symptoms and use of pain medication at start of
study. A study-related factor included was the randomised treatment arm (doxycycline, clarithromycin plus hydroxychloroquine, or placebo).

**PRE-TREATMENT FUNCTIONING:**

Pre-treatment scores on the primary and secondary outcome measures of physical and mental HRQoL were assessed before randomization and trial-treatment.

**INDIVIDUAL CHARACTERISTICS:**

The following variables were assessed at pre-treatment as possible predictors of the treatment outcome:

- **Pre-treatment expectancies regarding symptom improvement:** To evaluate expectancies on symptom progression, six items assessed the degree to which participants expected that their symptoms would disappear in the upcoming period (e.g., ‘I think that my complaints will totally disappear during the upcoming 6 months’ and ‘I think that I will no longer need any medical help for my complaints in the future’), in line with previous studies measuring pre-treatment expectancies as predictor of treatment outcome. Items could be answered on a 4-point Likert scale, varying from 1 ‘largely disagreed’ to 4 ‘largely agreed’, with a sum score range between 6 and 24. A higher total score indicates higher expectancies of improvement regarding the course of symptoms. The internal consistency of this measure was good, with a Cronbach’s alpha of .88.

- **Self-efficacy:** To determine the self-efficacy of participants, six statements on arthritis self-efficacy were adapted to a Lyme Self-Efficacy scale (LSE), in which the word ‘pain’ was replaced by ‘physical symptoms’ (e.g., “I am certain that I can control my physical symptoms”). Items are answered on a 1 (‘totally disagree’) to 5 (‘totally agree’) Likert scale, summing up to a total score between 6 and 30, with higher scores indicating more self-efficacy. Cronbach’s alpha was .78.

- **Illness cognitions:** The illness cognitions of helplessness (e.g., “Because of my illness I miss the things I like to do most”), acceptance (“I have learned to accept the limitations imposed by my illness”), and perceived benefits (“Dealing with my illness has made me a stronger person”) were assessed by means of three 6-item scales of the Illness Cognition Questionnaire (ICQ). Items are answered on a 1 (‘not at all’) to 4 (‘completely’) Likert scale, adding up to a score between 6 and 24, with higher scores indicating higher levels of
helplessness, acceptance, or perceived benefits, respectively. Cronbach’s alphas were .87 for helplessness and acceptance, and .84 for perceived benefits.

**Worrying:** To assess worrying, the Penn-State Worry Questionnaire (PSWQ)\(^{42}\) was used, including 14 statements (e.g., “I know I shouldn’t worry about things, but I just can’t help it”) measuring the tendency, intensity, and uncontrollability of worrying on a scale of 1 (“not at all typical of me”) to 5 (“very typical of me”). Higher scores indicate more worrying. Cronbach’s alpha was .93.

**Personality:** By means of the Eysenck Personality Questionnaire,\(^{43}\) neuroticism (22 items, e.g., “Does your mood often go up and down?”) and extraversion (19 items, e.g., “Do you enjoy meeting new people?”) were assessed by means of yes/no answers. Higher scores indicate more neuroticism and extraversion and Cronbach’s alphas were .87 and .86, respectively.

Post-treatment, one additional expectancy variable was assessed:

**Post-treatment expectancies of presumed study medication:** At the end-of-treatment (week 14), when returning the study medication bottles, participants were asked what medication they thought they had received, with answering options ‘antibiotics’, ‘placebo’ or ‘do not know’. To make this factor analysable in regression equations, the variable was converted into ‘antibiotics’ (score 1) or ‘placebo/don’t know’ (score 0).

**DATA ANALYSIS**

In order to allow analyses to be comparable across outcome measures per assessment point, analyses were performed on the data set with complete data on all predictor and outcome measures, leading to a sample of 231 patients at end-of-treatment and 170 at follow-up. Descriptive statistics of the variables of interest for this study were computed and changes in HRQoL between the different assessment points were assessed by means of paired-samples t-tests. To determine which factors could potentially impact quality of life change, zero-order associations of demographic, disease-related, study-related, and individual characteristics with the outcome measures at week 14 and 52, controlled for pre-treatment HRQoL, were examined by means of analyses of covariance (categorical characteristics) or partial correlations (continuous characteristics). The demographic and disease-related characteristics sex, age, and use of pain medication, and the individual characteristic acceptance (of the ICQ) did not show significant zero-order associations with
any of the quality of life outcome measures at end-of-treatment (week 14) or follow-up (week 52), controlled for pre-treatment quality of life. This held both in the total group and in separate analyses for the two combined longer-term treatment arms and the shorter-term treatment arm. Also, in line with the main results of the PLEASE trial, the study-related variable treatment arm was not associated with any of the quality of life changes in outcome measures, also when the two longer-term treatment arms were combined (all p-values ≥ .37). Therefore, these variables were not included in the regression analyses, with the main analyses being conducted in the total group, followed by sensitivity analyses for the shorter-term treatment arm and the combined longer-term treatment arms.

To examine the relative contribution of expectancies and other individual characteristics on physical and mental HRQoL after antimicrobial therapy, separate hierarchical regression analyses were conducted per outcome measure (PCS, fatigue, MCS). In the first block, demographic, disease-, and study-related characteristics being associated with at least one of the outcomes in the zero-order analyses were included. In the second block, the pre-treatment score of the outcome measure was included to control for cross-sectional variance with the other predictor variables, enabling the prediction of changes in HRQoL from pre-treatment to end-of-treatment and follow-up. In the third block, individual characteristics that showed zero-order associations with at least one outcome measure were entered. In order to ensure the most parsimonious model testing, definitive model testing was performed with only those predictor variables that showed at least one significant predictive association across all regression analyses.

Although the power analysis was based on the main research question of the PLEASE trial, the smallest sample size of 170 patients indicated adequate power according to the rule of thumb of at least 10 participants per predictor variable. All analyses were conducted with SPSS 25 and significance was accepted at $p<.05$. 
RESULTS

DESCRIPTIVE STATISTICS
Table 1 depicts the demographic, disease-, and study-related characteristics, baseline, end-of-treatment, and follow-up scores on the primary and secondary outcome measures, and baseline scores on the pre-treatment individual characteristics of the patients with complete end-of-treatment or follow-up data. Across groups, all quality of life outcome measures showed significant HRQoL improvements from pre-treatment to end-of-treatment (14 weeks; all p-values < .001), with further improvement (physical component summary score, p = .02) or stabilisation of the improvement (fatigue, p = .09; mental component summary score, p = .46) at follow-up (52 weeks). Differentiating longer-term versus shorter-term treatment showed similar findings for fatigue and mental HRQoL, and a continued improvement vs. stabilisation in the physical component summary score in the combined longer-term treatment arms vs. the shorter-term treatment arm (p = .045 vs. .19). In Appendix 1, the associations between the pre-treatment individual characteristics and demographic and disease-related factors are described.

PRE-TREATMENT PREDICTORS OF QUALITY OF LIFE COURSE
Table 2 shows the main results of the hierarchical regression analyses in the total group examining the prediction of quality of life course after antimicrobial therapy based on those pre-treatment variables that correlated with the HRQoL at end-of-treatment (14 weeks) or follow-up (52 weeks). Having a partner, education level, Lyme-related symptom duration, helplessness, disease benefits, and extraversion did not significantly add to explaining the variance in any of the outcome measures. Therefore, to present the most parsimonious model, these variables were excluded from the final regression models.
Table 1. Descriptive statistics of the demographic, disease-, study-related, outcome, and individual characteristics of the patients with complete data at end-of-treatment (14 weeks, n=231) or follow-up (52 weeks, n=170)

<table>
<thead>
<tr>
<th>Variables</th>
<th>End-of-treatment sample (n=231)</th>
<th>Follow-up sample (n=170)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (mean (SD))</td>
<td>49.88 (11.70)</td>
<td>51.19 (11.55)</td>
</tr>
<tr>
<td>Sex (female) (n (%))</td>
<td>106 (45.9)</td>
<td>82 (48.2)</td>
</tr>
<tr>
<td>Steady partner (n(%))</td>
<td>201 (87.0)</td>
<td>149 (87.6)</td>
</tr>
<tr>
<td>Education level (n(%))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>1 (0.4)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Secondary</td>
<td>123 (53.2)</td>
<td>84 (49.4)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>107 (46.3)</td>
<td>85 (50.0)</td>
</tr>
<tr>
<td>Smoking (n(%))</td>
<td>56 (24.2)</td>
<td>33 (19.4)</td>
</tr>
<tr>
<td>Paid labour (n(%))</td>
<td>143 (61.9)</td>
<td>102 (60.0)</td>
</tr>
<tr>
<td><strong>Disease-related factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lyme-related symptom duration (years) (median (IQR))</td>
<td>2.47 (1.16-6.39)</td>
<td>2.26 (1.15-6.31)</td>
</tr>
<tr>
<td>Use of pain medication (n(%))</td>
<td>162 (70.1)</td>
<td>115 (67.6)</td>
</tr>
<tr>
<td><strong>Study-related factors (n(%))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment arm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone followed by doxycycline</td>
<td>65 (28.1)</td>
<td>47 (27.6)</td>
</tr>
<tr>
<td>Ceftriaxone followed by clarithromycin &amp; hydroxychloroquine</td>
<td>81 (35.1)</td>
<td>59 (34.7)</td>
</tr>
<tr>
<td>Ceftriaxone followed by placebo</td>
<td>85 (36.8)</td>
<td>64 (37.6)</td>
</tr>
<tr>
<td><strong>Health-related quality of life (mean (SD))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical HRQoL (physical component summary score, T-score)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>31.81 (7.47)</td>
<td>31.91 (7.50)</td>
</tr>
<tr>
<td>End-of-treatment (14 weeks)</td>
<td>36.21 (10.16)</td>
<td>36.14 (10.16)</td>
</tr>
<tr>
<td>Follow-up (52 weeks)</td>
<td>–</td>
<td>37.60 (11.38)</td>
</tr>
<tr>
<td>Physical HRQoL (Fatigue severity, CIS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>43.79 (10.09)</td>
<td>44.09 (9.71)</td>
</tr>
<tr>
<td>End-of-treatment (14 weeks)</td>
<td>37.17 (13.40)</td>
<td>37.07 (13.80)</td>
</tr>
<tr>
<td>Follow-up (52 weeks)</td>
<td>–</td>
<td>35.72 (14.66)</td>
</tr>
<tr>
<td>Mental HRQoL (mental component summary score, T-score)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>37.75 (9.57)</td>
<td>37.38 (9.36)</td>
</tr>
<tr>
<td>End-of-treatment (14 weeks)</td>
<td>41.42 (11.28)</td>
<td>41.51 (11.34)</td>
</tr>
<tr>
<td>Follow-up (52 weeks)</td>
<td>–</td>
<td>42.08 (11.60)</td>
</tr>
</tbody>
</table>
Table 1. (Continued)

<table>
<thead>
<tr>
<th>Individual characteristics (mean (SD))</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Expectancies regarding symptom improvement</td>
<td>16.12 (4.46)</td>
<td>16.25 (4.43)</td>
</tr>
<tr>
<td>Self-efficacy (LSE)</td>
<td>17.23 (5.36)</td>
<td>17.37 (4.99)</td>
</tr>
<tr>
<td>Illness cognitions (ICQ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helplessness regarding disease</td>
<td>13.50 (4.24)</td>
<td>13.52 (4.34)</td>
</tr>
<tr>
<td>Disease acceptance</td>
<td>13.87 (3.89)</td>
<td>14.02 (3.90)</td>
</tr>
<tr>
<td>Perceived disease benefits</td>
<td>11.64 (4.13)</td>
<td>11.69 (4.21)</td>
</tr>
<tr>
<td>Worrying (PSWQ)</td>
<td>41.91 (12.28)</td>
<td>42.16 (12.31)</td>
</tr>
<tr>
<td>Personality (EPQ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroticism</td>
<td>7.94 (5.16)</td>
<td>7.87 (5.24)</td>
</tr>
<tr>
<td>Extraversion</td>
<td>11.49 (4.63)</td>
<td>10.79 (4.50)</td>
</tr>
</tbody>
</table>

Note. CIS = Checklist Individual Strength – Fatigue severity subscale; EPQ = Eysenck Personality Questionnaire; ICQ = Illness Cognition Questionnaire; IQR: interquartile range; LSE = Lyme Self-Efficacy; PSWQ = Penn-State Worry Questionnaire; RAND SF-36 = RAND-36 Health Status Inventory; SD = standard deviation

For all physical and mental HRQoL measures (physical component summary score, fatigue severity, and mental component summary score at end-of-treatment and at follow-up), each separate block of pre-treatment demographic characteristics, pre-treatment functioning, and pre-treatment individual characteristics significantly added explained variance to the model, with a total explained variance between 40 and 56%, depending on outcome measure. The largest amount of variance (27-40%) was explained by pre-treatment functioning on that particular outcome measure. The demographic characteristics paid labour (predicting better physical and mental HRQoL) and smoking (predicting worse mental HRQoL, mainly at follow-up) added 5 to 12%. The pre-treatment individual characteristics added 5 to 11% to the explained variance (Table 2).

In the total group, pre-treatment expectancies regarding symptom improvement consistently predicted physical and mental HRQoL at end-of-treatment (14 weeks) on top of pre-treatment functioning, thus predicting actual HRQoL improvement. This effect was even stronger at one year after start of treatment (follow-up at 52 weeks). Less consistently than pre-treatment expectancies, the other individual pre-treatment characteristics predicted mental (higher self-efficacy, less worrying, and lower neuroticism) and physical (higher self-efficacy) HRQoL improvement (Table 2).
Table 2. Percentage of explained variance and standardized regression coefficients (95% confidence intervals) of predictors of physical and mental health-related quality of life at end-of-treatment (14 weeks, n=231) and follow-up (52 weeks, n=170) in the total group (shorter- and longer-term treatment arms combined)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Physical HRQoL</th>
<th>Fatigue severity (CIS)</th>
<th>Mental HRQoL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Physical component summary score (PCS)</td>
<td>Week 14</td>
<td>Week 52</td>
</tr>
<tr>
<td>Demographic characteristics</td>
<td>ΔR²</td>
<td>.12†</td>
<td>.08**</td>
</tr>
<tr>
<td>Paid labour</td>
<td>ΔR²</td>
<td>.17* (1.56;5.49)</td>
<td>.13* (.34;5.87)</td>
</tr>
<tr>
<td>Smoking</td>
<td>ΔR²</td>
<td>-.04 (-3.04;1.24)</td>
<td>-.08 (-5.52;1.15)</td>
</tr>
<tr>
<td>Pre-treatment HRQoL</td>
<td>ΔR²</td>
<td>.38†</td>
<td>.27†</td>
</tr>
<tr>
<td>Pre-treatment PCS/CIS/MCS</td>
<td>ΔR²</td>
<td>.52† (55.;86)</td>
<td>.36† (32.76)</td>
</tr>
<tr>
<td>Individual characteristics</td>
<td>ΔR²</td>
<td>.05†</td>
<td>.10†</td>
</tr>
<tr>
<td>Expectancies symptom improvement</td>
<td>ΔR²</td>
<td>.14** (.09;54)</td>
<td>.23† (27.92)</td>
</tr>
<tr>
<td>Self-efficacy (LSE)</td>
<td>ΔR²</td>
<td>.09 (-.04;38)</td>
<td>.16* (.05;69)</td>
</tr>
<tr>
<td>Worrying (PSWQ)</td>
<td>ΔR²</td>
<td>-.08 (-.17;04)</td>
<td>.04 (-.12;19)</td>
</tr>
<tr>
<td>Neuroticism (EPQ)</td>
<td>ΔR²</td>
<td>-.10 (-.45;05)</td>
<td>-.17* (-.76;004)</td>
</tr>
<tr>
<td>Total R²</td>
<td>ΔR²</td>
<td>.54†</td>
<td>.45†</td>
</tr>
</tbody>
</table>

Note. * p < .10, ** p < .05, † p < .01; percentage of explained variance (Δ and total R²), and standardized regression coefficients (β, (95% CI) were assessed by means of hierarchical regression analyses; predictors were included when at least one significant association was found in prior regression analyses including all predictors showing any significant zero-order association with any of the outcome measures at end-of-treatment (14 weeks) or follow-up (52 weeks); Abbreviations: CIS = Checklists Individual Strength; EPQ = Eysenck Personality Questionnaire; HRQoL = health-related quality of life; ICQ = Illness Cognition Questionnaire; LSE = Lyme Self-Efficacy scale; MCS = mental component summary score; PSWQ = Penn-State Worry Questionnaire; RAND-36 PCS = RAND SF-36 Health Status Inventory – physical component summary score.
## Table 3.

Percentage of explained variance and standardized regression coefficients (95% confidence intervals) of predictors of physical and mental health-related quality of life at end-of-treatment (14 weeks, n=231) and follow-up (52 weeks, n=170) in the shorter- (ST) versus longer-term (LT) treatment arms.

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>Physical component summary score (PCS)</th>
<th>Mental component summary score (MCS)</th>
<th>Physical HRQoL</th>
<th>Mental HRQoL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔR²</td>
<td>Rx</td>
<td>Week 14</td>
<td>Week 52</td>
<td>ΔR²</td>
</tr>
<tr>
<td>ST</td>
<td>LT</td>
<td>ST</td>
<td>LT</td>
<td>ST</td>
</tr>
<tr>
<td>ΔR²</td>
<td>0.04</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paid labour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔR²</td>
<td>0.08 (-1.33;5.32)</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔR²</td>
<td>-0.04 (-4.52;2.82)</td>
<td>-0.04 (-3.82;1.82)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment HRQoL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔR²</td>
<td>0.51 †</td>
<td>0.30 †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment PCS/CIS/MCS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔR²</td>
<td>0.49 (46.99)</td>
<td>0.36 (18.82)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individual characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔR²</td>
<td>0.12 (-10.25)</td>
<td>0.18 (-12.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expectancies symptom improvement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔR²</td>
<td>0.19 (12.98)</td>
<td>0.20 (14.37)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-efficacy (LSE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔR²</td>
<td>-0.08 (-8.20;5.63)</td>
<td>-0.08 (-8.40;3.90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worrying (PSWQ)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔR²</td>
<td>-0.08 (-10.25)</td>
<td>-0.16 (-10.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroticism (EPQ)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔR²</td>
<td>-0.08 (-10.35)</td>
<td>-0.16 (-10.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total R²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>LT</td>
<td>ST</td>
<td>LT</td>
<td>ST</td>
</tr>
<tr>
<td>ΔR²</td>
<td>0.60</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Notes.
- *p < .10, **p < .05, ***p < .01, p < .001.
- Percentage of explained variance (Δ and total R²), and standardized regression coefficients (β, (95% CI) were assessed by means of hierarchical regression analyses; predictors were included in case at least one significant association was found in prior regression analyses including all predictors showing any significant zero-order association with any of the outcome measures at end-of-treatment (14 weeks) or follow-up (52 weeks).
- Abbreviations: CIS = Checklists Individual Strength; EPQ = Eysenck Personality Questionnaire; HRQoL = health-related quality of life; ICQ = Illness Cognition Questionnaire; LSE = Lyme Self-Efficacy scale; MCS = mental component summary score; PSWQ = Penn-State Worry Questionnaire; RAND-36 PCS = RAND SF-36 Health Status Inventory – physical component summary score; Rx: longer-term (LT) or shorter-term (ST) antimicrobial therapy.
Sensitivity analyses for shorter-term and longer-term treatment arms are reported in Table 3. Stratification of the duration of antimicrobial treatment (shorter-term versus longer-term treatment) overall showed a similar pattern of associations, with pre-treatment expectancies being the most consistent predictor of HRQoL at end-of-treatment and follow-up in both groups (Table 3). Differences between treatment arms consisted of the lack of predictive value of the demographic characteristics in the shorter-term antimicrobial treatment arm as opposed to the longer-term treatment arms (1-4% vs. 9-18% explained variance) and the larger predictive value of pre-treatment mental HRQoL for end-of-treatment and follow-up mental HRQoL in the shorter-term than longer-term treatment arms (51-52% vs. 28-34% explained variance).

**POST-TREATMENT EXPECTANCIES OF PRESUMED STUDY MEDICATION BEING ASSOCIATED WITH QUALITY OF LIFE COURSE**

At end-of-treatment (14 weeks), more patients presumed to have received antibiotics (n=148, 64.1%) than placebo (n=32, 13.9%) during blinded randomised treatment; the remainder indicated not to know (n=51, 22.1%). A significant difference in presumed medication was found between the treatment arms, with a larger percentage of patients in the longer-term antibiotics group who presumed to have received antibiotics compared to the placebo group (p = .007), with no difference between the two longer-term groups (p = .12).

When post-treatment expectancies of presumed study medication was included as a post-treatment predictor in the hierarchical regression analyses in an additional block, the explained variance of quality of life at end-of-treatment significantly increased by 2 to 3%, with patients who thought to have received antibiotics showing improvements in all outcome measures at end-of-treatment (14 weeks; PCS: β = .14, p = .003, 95%CI 1.00;4.75; CIS: β = -.17, p < .001, 95%CI -7.34;-2.22; MCS: β = .17, p < .001, 95%CI 1.95;6.07). Post-treatment expectancies did not add significantly to physical or mental HRQoL at follow-up (52 weeks; PCS: p = .22; CIS: p = .24; MCS: p = .07). These effects were similar in the shorter-term and longer-term treatment arms for end-of-treatment (14 weeks), whereas post-treatment expectancies were significantly (or with a trend towards significance) associated with physical and mental HRQoL at follow-up in the longer-term treatment arms only (PCS: p = .86 vs .08; CIS: p = .78 vs .03; MCS: p = .70 vs .03 in the shorter-term vs. longer-term treatment arms).
DISCUSSION

The current study examined the role of expectancies regarding symptom improvement and other individual characteristics in predicting quality of life course after antimicrobial therapy for persistent symptoms attributed to Lyme disease. In addition to pre-treatment functioning, pre-treatment expectancies regarding symptom improvement and post-treatment expectancies on having received antibiotics were found to be consistent predictors of larger improvements in physical and mental HRQoL. Other individual characteristics, related to more generalized outcome expectancies, showed less consistent predictive associations. Thus, where the main outcomes of our PLEASE trial showed that longer-term antimicrobial therapy did not have added benefits over shorter-term treatment for the quality of life outcomes, the current study showed that expectancies regarding symptom improvement and received study medication are associated with symptom course after both shorter-term and longer-term antimicrobial treatment. These findings suggest that expectancies may be useful in clinical settings in this patient group to improve symptom course and treatment outcome, which is in line with the current upsurge of research into the clinical potential of optimization of placebo effects and minimization of nocebo effects.

The role of positive or negative expectancies, for example regarding symptom course or treatment outcome, has been studied mostly within the area of placebo research. In this research, it has for instance been shown that induction of positive expectancies by means of learning procedures such as conditioning and verbal suggestions leads to decreased experience of physical symptoms such as pain and itch. Placebo effects have traditionally mainly been examined in the context of placebo-controlled randomised trials to discriminate the ‘real’ treatment effect from other ‘random’ effects. Currently, however, evidence has been accumulating that indicates the large clinical potential of implementing the placebo effect into the clinic to optimize patient care. Situation- or treatment-specific expectancies have not often been examined as predictors of treatment outcome or symptom course in clinical trials up to now. This is in contrast to more generalized outcome expectancy characteristics, such as the tendency to have faith in one’s abilities to deal with adversities (i.e. self-efficacy), to worry about potential negative future events (i.e., worrying), and to experience negative emotional states and to view the world as threatening (i.e., neuroticism). The current study examined the relative predictive contribution of situation-
specific expectancies next to other potentially relevant individual characteristics, of which only these more generalized outcome expectancies were found to be relevant. The results clearly showed that situation-specific pre- and post-treatment expectancies were the most consistent predictors of quality of life course at end-of-treatment and, for pre-treatment expectancies, even more strongly at follow-up. Of the more generalized outcome expectancy characteristics, less consistent associations were found for self-efficacy regarding dealing with one's symptoms, worrying, and neuroticism. Although in line with previous studies in other chronic conditions, it indicates less consistent evidence regarding the potential predictive value of more generalized as opposed to situation-specific outcome expectancy characteristics. This agrees with the findings of our previous study on the predictive role of situation-specific and generalized outcome expectancies in response to an immune-related training program in healthy men. In this study, situation-specific outcome expectancies were found to be associated to clinical symptom report after endotoxin administration. The findings of the current study thus suggest the added value of both pre- and post-treatment expectancies in explaining individual differences in treatment success regarding symptom course or treatment outcome in patients with persistent Lyme-associated symptoms and possibly also other chronic conditions.

Patients with persistent symptoms attributed to Lyme disease report a high symptom burden and disability, and low quality of life. As prolonged antimicrobial therapy has not lead to improved treatment outcomes, it is relevant to find other ways to improve symptom course and treatment outcome for this patient group. Patients with persistent symptoms for whom there is no gold standard treatment, such as the patients in our study, will have a high chance of having been confronted with negative treatment experiences. These negative experiences will automatically and unintentionally lead to negative outcome expectancies regarding new treatments. To prevent further disappointment, health care professionals tend to be hesitant to induce any positive expectancies in their patients. However, as the current study illustrates, pre-treatment expectancies of symptom improvement are relevant predictors of quality of life in both the shorter- and longer-term treatment arms. This underscores the relevance of examining different ways to optimize expectancies in clinical practice to improve treatment outcomes in this high-burdened patient group, for example by means of enhanced doctor-patient communication and open-label placebo treatments in which patients are informed about receiving a placebo and its working mechanisms. That
the most consistent and long-lasting effects were found for the patients receiving longer-term antimicrobial therapy, especially regarding post-treatment expectancies, may reflect that patients did notice somehow whether or not they received longer-term antibiotics, which probably has impacted their expectancies. Alternatively it could be explained by the lower power in the shorter-term group, as the two longer-term treatment arms were combined in the analyses.

The current study extended on the main findings of the PLEASE trial by showing that the treatment-independent improvements in quality of life from pre-treatment up to one year after start of treatment are associated with pre-treatment expectancies regarding symptom improvement. Also, the current findings suggest a stronger association of treatment outcome with presumed antibiotic use compared to actual antibiotic use. The fact that the question on presumed medication use was merely asked immediately after treatment allows for a bidirectional interpretation of the findings (i.e., treatment improvements impacting on the belief that one has received antibiotics versus believing one has received antibiotics impacting on treatment outcomes). However, presumed medication use remained a significant, although less strong, predictor of outcomes up to one year after start of treatment (38 weeks after presumed medication assessment), specifically in the longer-term treatment arms. Thus, although longer-term antimicrobial therapy has not shown to be more effective than shorter-term treatment, our patients may ascribe positive expectancies towards this treatment, which are related to a more positive outcome. This suggests the relevance of optimizing patient expectancies before the start of new treatment, of course within ethical boundaries.

Strengths of the current study include the large sample size and rigorous RCT study design. Also, the inclusion of pre-treatment functioning in the regression analysis provides a more stringent test of the added value of individual characteristics in actually predicting the change in HRQoL from baseline to end-of-treatment or follow-up. Limitations include the self-report nature of all predictor and outcome measures, allowing potential response bias effects. Also, the difference in patient numbers at end-of-treatment and follow-up prevents direct comparability of findings and the lower power in the shorter-term compared to the combined longer-term treatment arms complicates the interpretation of differences in predictions between groups. Finally, the assessment of post-treatment expectancies brings
inherent interpretability problems due to its assessment being intertwined with outcome assessment.

To conclude, the present study adds to our previously published PLEASE trial outcomes in showing how patients’ pre-and post-treatment expectancies regarding improvement of persistent Lyme-associated symptoms can explain a more beneficial symptom course. It would be relevant to examine in future research how expectancies could be optimized in patients with persistent symptoms attributed to Lyme disease, for instance by enhanced doctor-patient communication, in order to improve symptom course and treatment effectiveness. These results underscore recommendations to 1) ascertain that patient pre-treatment expectancies are realistic and can be met, and 2) inform patients and clinicians about the role of expectancies and taking these into account in treatments and research trials.
Appendix 1.

Associations between the individual characteristics and demographic, disease-, and study-related factors at study start, controlled for pre-treatment HRQoL, in the completer sample at end-of-treatment (14 weeks, n = 231)

More expectancies of symptom improvement were found in patients with shorter Lyme complaints ($r = -.21$, $p = .002$) or with paid labour ($17.02 \pm 4.17$ vs. $14.66 \pm 4.55$ for paid vs. no paid labour, $p < .001$). A higher level of self-efficacy was found in patients who had a higher age ($r = .22$, $p = .001$) or did not use pain medication ($16.62 \pm 5.21$ vs. $18.67 \pm 5.46$ for medication vs. no medication use, $p = .008$). Of the illness cognitions, more helplessness related to the disease was experienced by patients who had a younger age ($r = -.24$, $p < .001$), were female ($14.11 \pm 4.26$ vs. $12.98 \pm 4.18$ for women vs. men, $p = .04$), did not have paid labour ($12.93 \pm 3.99$ vs. $14.42 \pm 4.50$ for paid vs. no paid labour, $p = .009$), smoked ($14.60 \pm 4.28$ vs. $13.15 \pm 4.18$ for smoking vs. not smoking, $p = .03$), or used medication ($14.07 \pm 4.05$ vs. $12.14 \pm 4.41$ for medication vs. no medication use, $p = .008$); more disease acceptance was reported by patients of a higher age ($r = .21$, $p = .001$) or longer Lyme complaints ($r = .15$, $p = .03$). More worrying and more neuroticism were experienced by patients of a younger age ($r = -.22$ and $-.26$, respectively, $p$-values $\leq .001$), and more neuroticism was reported in patients with a lower educational level ($7.12 \pm 4.72$ vs. $8.64 \pm 5.43$, $p = .03$, for tertiary vs. primary or secondary education). Finally, more extraversion was reported by patients with a steady partner ($11.80 \pm 4.46$ vs. $9.40 \pm 5.39$ for partner vs. no partner, $p = .03$) and in patients who received one of the longer-term antibiotic treatments ($12.02 \pm 4.51$ vs. $10.58 \pm 4.73$ for patients in the longer-term vs. shorter-term treatment arms, $p = .02$). All associations were similar in magnitude for the completer sample at follow-up (52 weeks, $n = 170$), except for the associations between disease acceptance and symptom duration, between helplessness and smoking, and between extraversion and treatment arm, which all became non-significant at follow-up ($p = .17$, .13, and .07, respectively).
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Expectancies as predictors of symptom improvement after antimicrobial therapy for persistent symptoms attributed to Lyme disease
Summary, general discussion, and future perspectives
SUMMARY

This thesis gives more insight into patients with persistent symptoms that are attributed to Lyme disease and the effect of antibiotic treatment on their symptoms.

In chapter 2, we describe the study protocol of our randomized double-blind placebo-controlled trial, the Persistent Lyme Empiric Antibiotic Study Europe (PLEASE). This 3-arm trial evaluates whether 2 weeks of ceftriaxone followed by 12 weeks of doxycycline (arm 1) or ceftriaxone followed by the combination of clarithromycin and hydroxychloroquine for 12 weeks (arm 2) leads to better outcomes than short-term therapy with ceftriaxone followed by placebo (arm 3). The primary outcome measure is health-related quality of life at the end of the treatment period at week 14, assessed by the physical-component summary score of the RAND-36 Health Status Inventory (RAND SF-36). Patients are also evaluated after 26, 40, and 52 weeks.

In chapter 3, the main results of the PLEASE trial are presented. Of the 281 patients who underwent randomization, 280 were included in the modified intention-to-treat analysis (86 patients in the doxycycline group, 96 in the clarithromycin–hydroxychloroquine group, and 98 in the placebo group). The SF-36 physical-component summary score did not differ significantly among the three study groups at the end of the treatment period; the score also did not differ significantly among the groups at subsequent study visits. In all study groups, the SF-36 physical-component summary score increased significantly from baseline to the end of the treatment period. It was concluded that in patients with persistent symptoms attributed to Lyme disease, longer-term antibiotic treatment did not have additional beneficial effects on health-related quality of life beyond those with shorter-term treatment.

Chapter 4 describes the results of a prospective economic evaluation, adhering a societal perspective, that was performed alongside the PLEASE study. Mean quality-adjusted life years and total societal costs per patient were not significantly different between randomization arms. For every willingness-to-pay threshold, the incremental net monetary benefits did not significantly differ from zero. We concluded that the longer-term treatments in the PLEASE study were similar with regard to costs, effectiveness and cost-effectiveness compared to shorter-term treatment in patients with borreliosis-attributed persistent symptoms after one year of follow-up. Given these results, especially if one would also take external costs associated with antibiotic resistance into account, the shorter-term treatment is the antibiotic regimen of first choice from an economical viewpoint as well.
Chapter 5 discusses the secondary neuropsychological outcomes of the PLEASE trial. Cognitive performance was assessed with an extensive neuropsychological test battery covering the five major cognitive domains episodic memory, attention/working memory, verbal fluency, speed of information processing, and executive function. Of the 281 patients randomized, 85% (n=239) were included as they displayed sufficient performance validity. At end of treatment, performance on none of the cognitive domains differed significantly between the treatment arms. At follow-up, no additional treatment effect or difference between groups was found at any time-point. Patients performed significantly better in several cognitive domains at week 14, 26 and 40 compared to baseline, but this was not specific to a treatment group. We concluded that a 2-week treatment with ceftriaxone followed by a 12-week regimen of doxycycline or clarithromycin/hydroxychloroquine did not lead to better cognitive performance compared to a 2-week regimen of ceftriaxone in patients with Lyme disease-attributed persistent symptoms.

In chapter 6, we compare the cognitive performance of patients with borreliosis-attributed persistent symptoms to a large age-, education- and sex-adjusted normative sample of the general population in the Netherlands, while taking performance validity into account. Of the 280 patients included in this observational study of baseline data from the PLEASE study, 239 displayed sufficient performance validity. Patients with insufficient performance validity felt significantly more helpless and physically fatigued, and less orientated. Furthermore, they were characterized by a lower educational level and less often had paid work. Only 2.9% of the 239 patients with sufficient performance validity could be classified as cognitively impaired after neuropsychological assessment. There was no association between subjective cognitive symptoms measured by self-report questionnaires, and objective impairments assessed by neuropsychological assessment. We concluded that only a small percentage of patients with borreliosis-attributed persistent symptoms have objective cognitive impairment, and performance validity should be taken into account in neuropsychological examinations of these patients. In our study population, self-report questionnaires were insufficiently valid to diagnose cognitive impairment.

Expectancies about symptom improvement or deterioration are reliable predictors of symptom progression and treatment outcomes in a broad variety of (non-)pharmacological studies and treatments. In chapter 7, we examine the role of expectancies and other individual differences in predicting treatment outcome and symptom course in the PLEASE study population. In this predictive study, only expectancies regarding symptom...
improvement and pre-treatment functioning were consistently associated with stronger physical, including fatigue, and mental HRQoL improvements at both end-of-treatment and follow-up. Assumption that one has received antibiotics vs. placebo was also associated with more improvement on all outcome measures. This study showed how patients’ expectancies regarding improvement of persistent Lyme-associated symptoms may be associated with a more beneficial symptom course.

**GENERAL DISCUSSION**

Lyme borreliosis is the most prevalent tick-borne disease in the northern hemisphere, and its incidence has increased considerably over the past decades. Most patients with manifestations of Lyme disease are successfully treated with antibiotic therapy. However, regardless of initial appropriate treatment, persistent symptoms may arise. There is still much unclear about these persistent symptoms attributed to Lyme disease, also called post–Lyme disease syndrome. For example, the underlying pathophysiology and the characteristics associated with persistent symptoms are yet unknown. Furthermore, their treatment remains controversial, as clinical guidelines recommend different treatment periods, and as randomized, clinical trials had not consistently shown whether prolonged antibiotic treatment is effective or not.

We found that patients with persistent symptoms attributed to Lyme disease have a poor quality of life, reflected by their low baseline RAND SF-36 scores. Although these patients also report a high level of cognitive complaints, their cognitive performance was not substantially different from the general population, as only 2.9% had objective cognitive dysfunction, as measured by neuropsychological tests at baseline.

Furthermore, our research showed that after 14 weeks of antimicrobial therapy, there was no additional clinical benefit compared to shorter-term treatment for patients presenting with persistent symptoms attributed to Lyme disease such as fatigue, musculoskeletal, neuropsychological, or cognitive disorders. Although the quality of health at follow-up remained below that of the general population, it had improved significantly at end of treatment (after 14 weeks) compared to baseline, and remained better than baseline at one year of follow-up. However, this improvement was regardless of randomized treatment arm.
Whether this improvement was a beneficial effect of the unblinded ceftriaxone therapy, or a nonspecific effect caused by regression to the mean, spontaneous improvement over time, treatment expectancies, placebo effects, or a combination of these, remains unclear, as all patients had received 2 weeks of open-label antibiotics before entering into the placebo-controlled longer-term treatment phase. In retrospect, an evaluation moment directly after end of ceftriaxone treatment would have been valuable to obtain more clarity about this. However, our study on the role of outcome expectancies does suggest that expectancies, next to pre-treatment quality of life, have an important role in predicting outcome and symptom course.

Prior to implementation of a new therapeutic regimen such as longer-term antibiotic treatment, information about cost-effectiveness is important for rational decision making. Therefore, we planned a cost-effectiveness study alongside the trial, regardless of the primary outcomes of the PLEASE trial. After analysis of the primary outcome, we did not expect large differences in cost-effectiveness between longer-term and shorter-term antimicrobial treatment, as there was no additional clinical benefit of longer-term compared to shorter-term antimicrobial treatment on health-related quality of life, and oral antibiotic treatment is low-priced. Indeed, no significant differences in cost-effectiveness were found between treatment arms. Moreover, it is expected that the longer-term antibiotic treatment would have been less favorable if we could have taken the potential long-term effects of antibiotic overuse at a population level into account in our analyses.

One of the potential limitations of our studies is the absence of a full placebo arm. All patients received 2 weeks of open label antibiotic therapy preceding the randomized treatment phase. We chose to provide all patients with a standard treatment of ceftriaxone intravenously for 2 weeks, to also cover any potentially undiagnosed persistent infection, including neuroborreliosis. It was judged unethical to withhold treatment from patients who might have an untreated infection. Furthermore, by treating all patients, we also controlled for the wide variation in prior antibiotic therapies (or lack thereof) that patients with borreliosis-attributed persistent symptoms may have received, and for the variation of elapsed time between prior treatment and inclusion. Thus, the study was designed to compare longer-term to shorter-term therapy, as both approaches have been advocated by different position papers, rather than prolonged therapy to placebo. Consequently, the question whether 2 weeks of antibiotics is superior to withholding any therapy in our study
population remains unanswered. However, we did not set out to answer this question, as there has been no rationale that retreatment with antibiotics for 2 weeks would be helpful in curing long-term persistent symptoms attributed to Lyme disease. Rather, the discussion in the field was mainly whether longer-term antibiotics were effective for treating those persistent symptoms.

Also, the type and duration of antimicrobial treatment that was chosen for our trial merits consideration. For our randomized, 3-arm study we chose to compare a 12 weeks’ course of doxycycline to 12 weeks of clarithromycin and hydroxychloroquine versus 12 weeks of placebo, after an identical regimen of 2 weeks of ceftriaxone for all patients. Our choices were based on several considerations. First, ceftriaxone, followed by doxycycline if required, is generally considered the standard therapy for complex forms of Lyme borreliosis. Although ceftriaxone for longer than 2 weeks has been suggested, a randomized, open-label study was unable to demonstrate that ceftriaxone treatment for 4 weeks was significantly better than for 2 weeks. Secondly, a large case series of patients with borreliosis-attributed persistent symptoms, although uncontrolled and observational, suggested that prolonged therapy with oral doxycycline or other tetracyclines was successful. Data from another case series suggested that treatment with oral clarithromycin in combination with hydroxychloroquine for at least 3 months may be at least as effective as prolonged doxycycline for persistent symptoms attributed to Lyme disease. While, potentially, other classes of antibiotics may also be effective, these two antibiotic regimens were the only ones on which any observational data were published. For none of the other antibiotic regimens recommended by non-evidence-based guidelines, any pre-clinical or observational data has been published. Therefore, we chose to investigate the two therapies that were suggested in uncontrolled open label observational case series. Finally, it may be argued that 14 weeks of treatment is not long enough to obtain a treatment effect. Although extended antibiotic therapy is customary for various infectious diseases, prolonged therapy for infections is not required because of delayed onset of clinical alleviation but to prevent microbiological relapse. We are not aware of any infectious disease where the initial effect on symptoms, signs, and laboratory findings only begins after 3 months of effective therapy. In line with this, the uncontrolled studies by Donn et al. reported that almost 75% of patients with chronic Lyme disease improved within 1 month and 92% on tetracyclines or 100% on macrolides within 3 months of treatment.
Some may argue that the population that we included in our study was having complaints for too long to expect any improvement. However, the median duration of symptoms was 2.5 years, and almost 25% of patients had complaints for less than 14 months. This is relatively short, taking into account that a minimum of 6 months’ duration is required for the definition of post-Lyme disease syndrome. Furthermore, imbalance of characteristics between study arms was prevented through balancing randomization for several characteristics, including duration of symptoms (< or ≥ 1 year).

Missing data is a potential limitation in prospective studies, and our trial was no exception. The primary outcome data were missing for 7% of patients. This is relatively low compared to other trials. However, at follow up for secondary outcomes, this percentage increased, presumably because patients had to self-report many of the data. To reduce the influence of missing values, we performed an intention-to-treat analysis, and imputed missing data according to the baseline value-carried-forward method for the primary outcome. For the mixed-model analyses, missing data were imputed with the nearest available observation. Sensitivity analyses with a total of 4 alternate imputation techniques for missing data yielded equivalent outcomes. In our economic evaluation, we imputed data according to three scenarios (nearest available observation, worst-case and best-case scenario). Although all scenarios gave slightly different estimates, similar conclusions could be drawn, and our results were considered robust. Specific to the economic evaluation, some inaccuracies may have occurred due to costs that may not have been attributable to Lyme disease. For various episodes of healthcare utilization, it was difficult to attribute the costs to Lyme disease with certainty, as persistent symptoms can be non-specific. Nevertheless, our study population represents the patients who are actually encountered in clinical practice, who do suffer from low quality of life, and have high healthcare consumption and productivity losses.

The present studies have several strengths. The most important asset is the fact that we performed a double-blind randomized placebo-controlled trial. This type of study is regarded as the highest level of scientific research, as it aims at measuring purely the effect of the treatment without bias by other factors. While previous trials on prolonged antibiotic therapy for Lyme disease had included 37 to 145 patients, our study with 280 patients is the largest so far. Importantly, there has been discussion about the clinical relevance of the cut-offs used for the primary outcome in previous trials. For our trial, we performed a pilot study to determine the clinically relevant treatment effect, which was used to interpret the subsequent randomized trial data.
Furthermore, we put specific efforts into ensuring patients’ compliance with study medication. This included a medication event monitoring system, which showed that only 8% of patients were recorded to have discontinued treatment for more than a week prior to the primary endpoint.

For the cost-effectiveness part of the study, we prospectively collected data on health states, and costs were on patient level. This made it possible to provide relatively precise estimates. Moreover, we performed our analyses from a societal perspective, since we included productivity losses and travel expenses as cost categories as well. That is considered optimal, as it includes all relevant societal costs and benefits irrespective of who bears or accrues them.

In the neuropsychology part of our study, we were the first to take suboptimal cognitive effort into account, when assessing the cognitive functioning in patients with persistent symptoms attributed to Lyme disease. This was done by selecting only those patients who performed well on the Amsterdam Short Term Memory Test. This test is a simple test, but appears to be difficult. Poor performance on this task indicates suboptimal mental effort. A suboptimal performance results in poor tests scores that do not indicate the actual cognitive status of the subject. Furthermore, we assessed neurocognitive function in the largest sample of patients with post-treatment Lyme disease so far, and were the first to relate their outcomes to extensive normative data.

Finally, our study results are considered generalizable as we included exactly those patients that generally visit outpatient clinics with persistent symptoms attributed to Lyme disease.

**FUTURE PERSPECTIVES**

The research in this thesis focuses on patients with persistent symptoms attributed to Lyme disease and antibiotic treatment of their symptoms. We showed that it is not effective to treat these patients with longer-term antimicrobial treatment of doxycycline or clarithromycin/hydroxychloroquine for 12 weeks after 2 weeks of ceftriaxone, compared to short-term treatment with ceftriaxone alone. Although there is neither evidence nor expectation that treatment with antibiotics for 2 weeks would cure long-term persisting symptoms
attributed to Lyme disease, the exact role of ceftriaxone in our study remains unknown, as all randomization arms improved but also all had received ceftriaxone. If more clarity would be required on this issue, it would be necessary to perform a treatment trial with a full placebo arm. When designing our study, we felt that it was unethical to have a full placebo arm as we might include patients with untreated infection. However, our study showed that there was no difference in outcomes after longer-term placebo or antibiotic treatment, suggesting that there was little untreated Lyme infection, clearing the way for a full placebo arm in future research.

Furthermore, a pilot investigation with other antibiotic regimens than the ones we used could be considered. However, before initiating new clinical trials, a rationale for the potential effectiveness of new therapies should be provided by in vitro studies and case series.

Also, exploring non-pharmacological treatment as a therapy for persistent symptoms would be interesting, e.g. cognitive behavioral therapy to tackle the fatigue and cognitive symptoms experienced by most patients with persistent symptoms attributed to Lyme disease. Cognitive behavioral therapy has proven to be effective in reducing chronic fatigue in both chronic fatigue syndrome and in chronic somatic illnesses 17-21. As patients might already benefit from the intervention process, it would be most valuable to compare cognitive behavioral therapy to a sham therapy.

We studied the effect of antibiotic treatment on persistent symptoms attributed to Lyme disease. However, there is still much to be discovered about these persistent symptoms. This includes their presence, as the reported prevalence of persistent symptoms varies highly, from 0 to 48% 22. In 2015, the LymeProspect study was set up to obtain more information about the prevalence and severity of persistent symptoms. This multi-center, prospective, observational cohort study was initiated by the Netherlands Lyme Disease Center of Expertise (NLe), a collaboration between the Radboudumc, Amsterdam UMC, RIVM, and patients’ representatives 22. Patients with proven or probable Lyme disease (erythema migrans or disseminated borreliosis) are included at the start of antibiotic treatment, and are followed for one year. Control cohorts include patients with long-lasting symptoms and unconfirmed Lyme disease, population controls, and subjects having reported a tick bite that was not followed by Lyme borreliosis.
Furthermore, besides obtaining more insight into the magnitude of persistent symptoms attributed to Lyme disease, it is also important to define whether symptoms are truly attributed to Lyme disease. That is, it is imperative to rule out other reasons for persistent symptoms, but also to demonstrate an active *Borrelia burgdorferi* infection with more certainty. For this we need better tests than the present serology. In our laboratory for experimental internal medicine, a cellular test has been developed that may discriminate between an old (treated) or active *Borrelia* infection. This and other tests are currently being validated in the Victory study, another initiative by the Netherlands Lyme Disease Center of Expertise.

In this thesis, we reported about the quality of life of patients with persistent symptoms attributed to Lyme disease until one year after treatment. However, there still is little known about the long-term course in these patients. On an individual basis, patients report improvement after longer-term antibiotic treatment or other non-standard therapies. As baseline characteristics, including quality of life, of the patients from the PLEASE trial are well documented, it would be valuable to study the long-term course of disease in this cohort. What is their quality of life from five years after treatment? Which additional therapies have these patients undergone, and what has contributed to a possible improvement? Also, the long-term impact of persistent symptoms on occupational disability has not been investigated before. Recently, the ZonMw Medical Inspirator prize has been assigned to the PLEASE investigators to investigate these issues.
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Nederlandse samenvatting

Dankwoord

List of publications and lectures

Curriculum vitae
NEDERLANDSE SAMENVATTING

In de noordelijke hemisfeer is de ziekte van Lyme de meest voorkomende door teken overgedragen ziekte, en de incidentie is de laatste jaren verder toegenomen. Deze infectieziekte wordt veroorzaakt door de bacterie *Borrelia burgdorferi*, die onder andere de huid, de gewrichten of het zenuwstelsel kan infecteren. De meeste patiënten met uitingen van Lymeziekte kunnen succesvol worden behandeld met antibiotica. Er kunnen echter, ondanks de juiste initiële behandeling, persistierende symptomen ontstaan. Er is nog veel onduidelijk over deze persistierende Lyme-geassocieerde klachten, ook wel post-Lymeziekte syndroom genoemd. Zo zijn de onderliggende pathofysiologie en de karakteristieken waarmee de persistierende symptomen zijn geassocieerd, nog onvoldoende bekend. Daarnaast blijft de behandeling van deze klachten controversieel omdat klinische richtlijnen een verschillende behandelduur adviseren, en gerandomiseerde klinische trials niet consistent hebben aangetoond of langdurige antibioticabehandeling effectief is. Dit proefschrift geeft meer inzicht in patiënten met persistierende klachten die toegeschreven worden aan de ziekte van Lyme, en het effect dat behandeling met antibiotica heeft op hun klachten.

In *hoofdstuk 2* beschrijven we het studieprotocol van onze dubbelblinde gerandomiseerde placebo-gecontroleerde trial, de “Persistent Lyme Empiric Antibiotic Study Europe” (PLEASE). In dit type onderzoek bepaalt het lot welke behandeling een patiënt krijgt (randomiseren), zodat de indeling in groepen eerlijk gebeurt, en het succes van de behandeling niet wordt beïnvloed door ongelijke groepen. De behandeling is dubbelblind, hetgeen wil zeggen dat tijdens het onderzoek zowel de patiënt als de onderzoeker niet weten welke behandeling gegeven is. Dit wordt gedaan om (onbewuste) beïnvloeding van de onderzoeksresultaten, die ontstaan door verwachtingen, te voorkomen. Hiervoor wordt een placebo middel ingezet, dit middel is identiek aan het antibioticum maar bevat geen werkzame stof. Onze 3-armige trial evalueert of 2 weken ceftriaxon gevolgd door 12 weken doxycycline (arm 1), dan wel ceftriaxon gevolgd door de combinatie van claritromycine en hydroxychloroquine gedurende 12 weken (arm 2), leidt tot betere uitkomsten dan kortdurende behandeling met 2 weken ceftriaxon gevolgd door placebo (arm 3). De primaire uitkomst is de gezondheidsgerelateerde kwaliteit van leven aan het einde van de behandelingsperiode op week 14, bepaald door middel van de lichamelijke component.
score van de RAND-36 Health Status Inventory (RAND SF-36), een vragenlijst. Daarnaast zijn er meetmomenten op 26, 40, en 52 weken na start van de behandeling. De gebruikte analyse is een “intention-to-treat” analyse. Dit betekent dat de patiënten geanalyseerd worden in de groep waarin ze oorspronkelijk zijn ingedeeld, waarbij het niet uitmaakt of ze de behandeling uiteindelijk niet volledig naleven. Hiermee kan de doeltreffendheid van de behandeling beter beoordeeld worden omdat dit de dagelijkse praktijk weerspiegelt.

In hoofdstuk 3 beschrijven we de hoofdresultaten van de PLEASE trial. Van de 281 patiënten die gerandomiseerd werden, zijn er 280 opgenomen in de gemodificeerde “intention-to-treat” analyse (86 patiënten in de doxycycline groep, 96 in de claritromycine-hydroxychloroquine groep, en 98 in de placebo groep). De lichamelijke component score verschilde niet significant tussen de 3 groepen aan het einde van de behandelingstijd; deze score was evenmin significant verschillend tussen de groepen op de opeenvolgende evaluatiemomenten. Ten opzichte van de nulmeting, was de lichamelijke component score in alle groepen significant beter aan het einde van de behandelingstijd. We concludeerden dat bij patiënten met persisterende Lyme-geassocieerde klachten een langdurige behandeling van 3 maanden met de meest gebruikte antibiotica geen additioneel gunstig effect heeft op gezondheidsgerelateerde kwaliteit van leven vergeleken met kortdurende behandeling.

Hoofdstuk 4 beschrijft de resultaten van een prospectieve economische evaluatie, vanuit maatschappelijk perspectief. Deze evaluatie werd verricht tegelijkertijd met de PLEASE trial. Het gemiddelde aantal levensjaren gecorrigeerd voor kwaliteit van leven (QALYs) en totale maatschappelijke kosten per patiënt waren niet significant verschillend tussen de 3 groepen. Voor elke drempel ten aanzien van bereidheid om te betalen voor gezondheidswinst, waren de “incremental net monetary benefits” niet significant verschillend van nul. “Incremental net monetary benefit” is een statistiek die de waarde van een interventie in geld weergeeft wanneer de drempel van betalingsbereidheid voor een QALY bekend is. We concludeerden dat bij patiënten met persistenende Lyme-geassocieerde klachten de langdurige antibioticabehandelingen van de PLEASE studie vergelijkbaar zijn met de kortdurende behandeling ten aanzien van kosten, effectiviteit en kosteneffectiviteit, gemeten tot een jaar na start van de behandeling. Gezien deze resultaten, en helemaal als men kosten zou kunnen meenemen die geassocieerd zijn met antibioticaresistentie, is de kortdurende behandeling van 2 weken de eerste keus vanuit economisch oogpunt.
Hoofdstuk 5 bespreekt de secundaire neuropsychologische uitkomsten van de PLEASE trial. Cognitief functioneren werd onderzocht door middel van een uitgebreide neuropsychologische testbatterij met de volgende 5 belangrijke cognitieve domeinen: geheugen, aandacht/werkgeheugen, taal/woordvloeiendheid, snelheid van informatieverwerking, en uitvoerende functies. In deze studie werden alleen patiënten die niet onderpresteren geïncludeerd; dit waren 239 van de 281 gerandomiseerde patiënten van de PLEASE trial (85%). Onderpresteren werd gemeten door middel van de Amsterdamse Korte Termijn Geheugen test. Dit is een test die erg moeilijk lijkt maar dat in feite niet is; zelfs mensen met hersenletsel kunnen de test goed maken. Aan het einde van de behandeling was het functioneren in geen enkel cognitief domein significant verschillend tussen de behandelgroepen. Tevens werd bij het vervolgen van de patiënten op geen enkel evaluatiemoment een additioneel behandeleffect of een verschil tussen de groepen gezien. Patiënten presteerden significant beter in enkele cognitieve domeinen op week 14, 26 en 40 vergeleken met de nulmeting, maar dit was ongeacht of patiënten langdurig of kortdurend behandeld werden. We concludeerden dat een behandeling met 2 weken ceftriaxon gevolgd door 12 weken doxycycline of claritromycine/hydroxychloroquine niet leidt tot beter cognitief functioneren vergeleken met een behandeling van alleen 2 weken ceftriaxon in patiënten met persisteerende Lyme-geassocieerde klachten.

In hoofdstuk 6 vergelijken we het cognitief functioneren van patiënten met persisteerende Lyme-geassocieerde klachten met een grote normatieve steekproef van de algemene Nederlandse populatie (aangepast aan leeftijd, opleiding en geslacht), waarbij rekening werd gehouden met onderpresteren. Voor deze observationele studie werden de gegevens gebruikt van de patiënten van de PLEASE studie voor aanvang van de antibiotiebehandeling met ceftriaxon. Van de 280 patiënten die geïncludeerd werden lieten 239 patiënten voldoende inzet zien om de testen verder te kunnen analyseren. Er was geen relatie tussen subjectieve cognitieve klachten (gemeten door middel van vragenlijsten die door de patiënten zelf werden ingevuld) en subjectieve verslechtering (gemeten door middel van neuropsychologisch onderzoek). Slechts 2,9% van de 239 patiënten met voldoende inzet konden na neuropsychologisch onderzoek geclassificeerd worden als cognitief beperkt. Patiënten die onderpresteren, voelden zich significant meer hulpeloos en lichamelijk moe, en minder georiënteerd. Tevens werden ze gekarakteriseerd door een lager opleidingsniveau en hadden ze minder vaak betaald werk. We concludeerden dat slechts
een klein percentage patiënten met persisterende Lyme-geassocieerde klachten cognitieve beperkingen in het neuropsychologisch onderzoek hadden, en dat er rekening gehouden moet worden met mogelijk onderpresteren bij neuropsychologisch onderzoek van patiënten met Lyme-geassocieerde klachten.

Verwachtingen over symptoomverbetering en -verslechtering zijn betrouwbare voorspellers van progressie van symptomen en behandeluitkomsten in een breed scala aan farmacologische en niet-farmacologische onderzoeken en behandelingen. In hoofdstuk 7 onderzoeken we in de PLEASE-onderzoekspopulatie de rol van verwachtingen en andere individuele verschillen in het voorspellen van behandeluitkomst en symptoombeloop. In deze voorspellende studie waren alleen beter functioneren vóór de behandeling en positievere verwachtingen betreffende symptoomverbetering consistent geassocieerd met sterkere verbetering in lichamelijke (inclusief moeheid) en mentale gezondheidsgerelateerde kwaliteit van leven, gemeten aan het einde van de behandelingsperiode en een jaar na start van de behandeling. De aannames van de deelnemers dat men antibiotica in plaats van placebo had ontvangen was ook geassocieerd met meer verbetering bij alle uitkomstmaten. Deze studie laat zien hoe de verwachting van een patiënt betreffende verbetering van persisterende Lyme-geassocieerde klachten wellicht gerelateerd is aan een gunstiger symptoombeloop.

Kortom, dit proefschrift toont aan dat patiënten met persisterende Lyme-geassocieerde klachten een matige kwaliteit van leven ervaren. En hoewel ze tevens veel cognitieve klachten rapporteren, vonden we bij objectief neuropsychologisch onderzoek geen aanwijzingen voor een verminderd cognitief functioneren ten opzichte van de algemene populatie. Verder laat dit proefschrift zien dat er na 14 weken antibiotische behandeling geen klinische verbetering was ten opzichte van behandeling met 2 weken antibiotica bij patiënten met persisterende Lyme-geassocieerde klachten zoals moeheid, gewrichtsklachten, of cognitieve problemen. Evenmin vonden we een verschil in kosten-effectiviteit tussen de verschillende behandelingen.
Mijn promotietraject:
vaak Stormde het,
soms voelde ik Vlinders in mijn buik,
en uiteindelijk Meanderde het naar een goed einde.
Allen bedankt!
LIST OF PUBLICATIONS


Berende A, Tacken MA, de Bakker DH, Hak E, Brasperning JC. Eerdere influenzavaccinatie voor hoogrisicopatiënten die vaker contact met hun huisartspraktijk hebben. *TSG: Tijdschrift voor Gezondheidswetenschappen.* 2004;82(8),512-517.

LECTURES

- Internistendagen, 2017, Maastricht, Plenaire sessie Toppublicaties, Randomized Trial of Longer-Term Therapy for Symptoms Attributed to Lyme Disease
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CURRICULUM VITAE


Anneleen is getrouwd met Hans, en samen hebben zij drie bloedmooie kinderen: Storm, Vlinder, en Meander.
Persistent symptoms attributed to Lyme disease and their antibiotic treatment

Results from the PLEASE study

- Anneleen Berende -