CLINICAL PROTOCOL

PHASE III

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTICENTER TRIAL OF THE SAFETY AND EFFICACY OF CEFTRIAXONE AND DOXYCYCLINE IN THETREATMENT OF PATIENTS WITH SEROPOSITIVE CHRONIC LYME DISEASE

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I. INTRODUCTION

Lyme disease (LD) is the most common tick-borne disease in the United States.[1] The etiologic agent, *Borrelia burgdorferi*, is a spirochete, and is transmitted to humans and other animals by tick vectors belonging to the genus Ixodes. The natural reservoir for the etiologic agent is rodents; many other types of mammals and some birds may also become infected [2].

As with many infectious diseases, the clinical manifestations of LD are variable and unpredictable. Early manifestations include a rash (erythema migrans), general malaise, and flu-like symptoms [3]. Chronic manifestations include arthritis, cardiac and neurologic manifestations that have been reported to spontaneously remit and recur even following antibiotic therapy [4 5 6 7 8 9].

Recently, the term Chronic Lyme Disease has been used to describe a condition of chronic or intermittent symptoms related to LD. The cause of CLD is not known, but several possibilities have been suggested. The first is that it is the manifestation of a chronic active infection by *B. burgdorferi* that has escaped control or eradication with the use of conventional antibiotic regimens [10 11] . A second possibility is that CLD may be due to damage caused by the original infectious process, including triggering of post-infectious immune phenomena, despite the eradication of the spirochete[12]. A third possibility is the presence of a co-infection with another organism transmitted by Ixodes ticks.

II. OBJECTIVES

The objectives of this study are to determine whether: 1) intensive antibiotic treatment benefits seropositive patients with CLD; 2) evidence of persistent infection with Borrelia burgdorferi can be found in patients with CLD; 3) evidence of co-infection with other microorganisms can be found in patients with CLD; 4) specific clinical or laboratory

parameters improve in patients who receive antibiotic therapy compared to patients who receive placebo and 5) specific parameters are predictive of a response to therapy should it be observed.

III. DESIGN AND STUDY DESCRIPTION

This study, which is being supported as contract through funds made available by the NIAID, is a Phase III, randomized, double-blinded, placebo-controlled, multicenter trial (two centers). One hundred ninety four (194) patients will be enrolled in the study. Each patient will be assigned to one of three strata based upon the duration of symptoms of chronic Lyme disease (less than four years, four to less than eight years, or eight to less than twelve years), and randomized to receive either antibiotic therapy or placebo in a 1:1 ratio. Antibiotics and placebo will be administered both intravenously and orally. Separate randomization schedules will be generated for each study center by NIAID or its designate. The study population will include a defined cohort of patients with CLD who meet the inclusion and exclusion criteria as defined for this study (see section V).

The antibiotic regimen will be ceftriaxone, 2.0 grams/ day, administered once daily via the intravenous route for 30 consecutive days followed by doxycycline, 200 mg/day, administered as 100mg twice daily via the oral route for 60 consecutive days. Placebos identical to the intravenous and oral medications will be administered via the same route and for the same duration to the patients randomized to the placebo treatment group.

IV. MATERIALS AND SUPPLIES

A. Study Drugs

Intravenous ceftriaxone at a dose of 2 grams daily or intravenous placebo (dextrose) will be administered for the first 30 days of the treatment period to patients assigned to the active treatment group (Group A) and the placebo treatment group (Group B)

respectively. Patients in group A will then receive doxycycline 100 mg every 12 hours orally for 60 consecutive days and patients in group B will receive an identical placebo every 12 hours orally for 60 consecutive days. Dr. Mark Klempner or a physician co-investigator of New England Medical Center and Dr. Gary Wormser or a physician co-investigator of New York Medical College, will supervise all aspects of the administration of the study medications. Patients will receive intravenous therapy at home with an indwelling short plastic venous access catheter.

There will be two groups in this study with 97 patients in each group:

1. IV ceftriaxone x 30 days followed by oral doxycycline x 60 days

2. IV placebo (dextrose) x 30 days followed by oral placebo (dextrose) x 60 days

V. PATIENT SELECTION AND ENROLLMENT

A. Study Population

This study will be conducted in 194 patients with CLD. Enrollment will begin in approximately April 1997 and proceed until the quota of patients is reached. We anticipate an enrollment period not to exceed three years.

B. Inclusion and Exclusion Criteria

For the double-blind, placebo-controlled trial of antibiotics vs. placebo in seropositive patients with chronic Lyme disease, the following criteria for inclusion will be used.

Inclusion criteria:

1. IgG seropositivity at the time of enrollment into the study for an immune response to *B. burgdorferi* antigens according to the currently accepted CDC (Dearborn) criteria defined in reference [13] .

2. 18 years of age or older.

3. Able to give informed consent.

4. Physician documented history of prior antibiotic treatment with a currently recommended antibiotic regimen that was appropriate for the patient's clinical features of Lyme disease at the time of presentation (table on page 8 of the technical proposal).

5. A past history of one or more of the following clinical features typical of Lyme disease acquired in the United States:

   a) A past history of erythema migrans defined as an erythematous skin lesion that expands over a period of days to weeks to form an annular lesion.

   b) Multiple erythema migrans lesions indicative of disseminated disease.

   c) Early neurologic disease that includes lymphocytic meningitis, cranial neuritis (e.g. facial palsy), or radiculoneuropathy not attributable to other causes.

   d) Acute cardiac illness consisting of signs and symptoms associated with various degrees of A-V block not attributable to other causes.

e) Lyme arthritis defined as recurrent, brief attacks of objective joint swelling in one or a few joints, especially the knees, sometimes followed by chronic monoarthritis not attributable to other causes.

6. One or more of the following symptoms that have persisted for at least 6 months and are not attributable to another cause or condition:

a) Widespread musculoskeletal pain and fatigue that interferes with usual function and which began coincident with or within 6 months following initial infection with *B. burgdorferi*.

b) Symptoms of memory impairment that interfere with usual function and which began coincident with or within 6 months following initial infection with *B. burgdorferi*.

c) Symptoms of radicular pain, paresthesias and/or dysesthesias that interfere with usual function and which began coincident with or within 6 months following initial infection with *B. burgdorferi*.

Exclusion criteria:

1. A history of hypersensitivity to ceftriaxone or doxycycline.

2. Currently, or within the last 7 days, taking beta lactam, tetracycline or macrolide antibiotics.

3. Previously received a total of $\geq$ 60 days of parenteral ceftriaxone or cefotaxime therapy for:

a) Widespread musculoskeletal pain and fatigue that interferes with usual function and which began coincident with or soon after initial infection with *B.burgdorferi*.

b) Symptoms of memory impairment that interfere with usual function and which began coincident with or soon after initial infection with *B. burgdorferi*.

c) Symptoms of radicular pain that interfere with usual function and which began coincident with or soon after initial infection with *B. burgdorferi*.

4. Having received $\geq$ 14 days of parenteral ceftriaxone or cefotaxime therapy within the last 60 days.

5. Patients with active inflammatory synovitis.

6. Patients whose symptoms of CLD (a, b, c above) have been present for $\geq$ 12 years.

7. Patients who have comorbid disease(s) that could account for symptoms of chronic Lyme disease (a, b, and c above). Examples include severe clinical depression, rheumatic illness such as rheumatoid arthritis or SLE, other potential causes of radiculopathic pain such as intervertebral disc disease, etc.

8. Patients who have a serious comorbid disease (e.g. hematologic malignancy, cirrhosis, metastatic cancer, etc.) or an active infection (e.g., HIV, tuberculosis, etc.).

9. Patients who are receiving chronic medication therapy that could interfere with the evaluation of symptoms in a, b, c above (e.g., narcotic analgesics, prednisone $\geq$ 10mg/day).

10. Patients who cannot tolerate or do not have adequate venous access for an indwelling venous access catheter or are at increased risk of acquiring an intravenous catheter related infection.

11. Patients who are pregnant, lactating, or unable to use birth control measures during the treatment period of the study.

12. Patients who have previously enrolled in this study.

13. Patients who have positive PCR for Borrelia DNA in plasma or cerebrospinal fluid at the time of initial evaluation for this study.

VI. STUDY PROCEDURES

A. Initial Evaluation Procedures (Up to Two Weeks Prior to Dosing)

Within four weeks prior to administration of antibiotics or placebo, the following will be obtained to assess compliance with the subject selection criteria outlined in Section V. Patients will have a complete history and physical examination including detailed neurologic examination and evaluation of tender points at 18 sites. Standardized history and physical examination will be used to assess each patient for collection of demographic data, a history of exposure to ticks in geographic regions with endemic *B. burgdorferi* infection, clinical manifestations of acute Lyme disease, clinical manifestations of chronic Lyme disease, prior treatment, history of previous serologic tests for Lyme disease (although serologic status will be determined in the central laboratory at the time of initial testing on entry to the study), medication allergies, recent (last 2 months) and current medications. If the patient meets all the inclusion criteria and none of the exclusion criteria, the study protocol will be explained and the patient will be offered participation in the study.

Patients who, upon initial evaluation, have (a) a positive PCR for Borrelia DNA in plasma or cerebrospinal fluid (CSF) or (b) evidence of synovitis will not be randomized (see exclusion criteria #13). Such patients will be referred to either the NIAID Intramural Clinical Study on Chronic Lyme Disease or elsewhere for appropriate treatment.

To determine whether spirochetes may persist in the skin of patients with chronic Lyme disease, a skin biopsy will be obtained at the initial erythema migrans site from a subset of up to 30 patients with a history of erythema migrans at New England Medical Center.

These specimens will be obtained from study subjects who volunteer to have the biopsy procedure performed. This procedure is not a mandatory part of the Seropositive protocol. There will be one biopsy performed per volunteer subject prior to initiating treatment.

Initial Evaluation:

1. Complete medical history (including all questions on data base forms related to manifestations of acute Lyme disease and to current symptoms of chronic Lyme disease).

2. Complete physical examination including detailed examinations of the musculoskeletal and neurologic systems.

3. Administration of the SF-36 Health Survey[14] and the Fibromyalgia Impact Questionnaire [15].

4. Vital signs - blood pressure, pulse rate, respiratory rates and temperature.

5. Lumbar puncture will be performed on all patients according to standard sterile procedures. CSF will be examined for cell count, total protein, glucose, production of antibody to *B. burgdorferi* antigens [16] and PCR for detection of DNA from *B.burgdorferi* [17] and Ehrlichia sp. [18].

6. Laboratory tests as follows: CBC and differential counts, ESR, ANA, total bilirubin, serum glutamic-oxaloacetic transaminase (SGOT/AST), lactate dehydrogenase (LDH), alkaline phosphatase, creatinine, blood urea nitrogen (BUN), total protein, glucose, albumin, TSH, PCR for detection of DNA from *B.burgdorferi* in serum, and total IgG. A CSF IgG index can be calculated according to the following formula: (CSF IgG x serum albumin)/(CSF albumin x serum IgG). The rate of IgG synthesis is calculated as follows: 5 x CSF IgG - IgG serum/68.2 2.15(CSF albumin—serum albumin/109) (serum IgG/serum albumin).

7. Neuropsychological testing: to provide measures of immediate and delayed memory, conceptualization, copying, perceptual discrimination, and language. Each of these tests will be administered at baseline, 90 days and 180 days except the MMPI that will be administered at baseline and 180 days only.

   a) Rey Auditory - Verbal Learning Test (RAVLT)
   Rationale: it is similar to those tests (i.e., California Verbal Learning Test[19], and Selective Reminding Test) shown sensitive to Lyme-related memory loss and it has several alternate forms. (time = 20 minutes)

   b) Symbol-Digit Modalities Test A test that requires attention and concentration in addition to visual perception.
   Rationale: shown to be sensitive by Halperin[20] to Lyme-related decline performance. (time = 2-3 minutes)

   c) MMPI A personality inventory and psychopathology screen.
   Rationale: previously used to compare Lyme and fibromyalgia patients by Kaplan [21] and may reveal differences in personality profile of chronic Lyme patient. [22] [23](time = 45 minutes)

   d) Beck Depression Inventory A depression index.
   Rationale: previously used in several Lyme studies to assess depression and compare depression to other patient groups. (time = 5-10 minutes)

   e) California Computerized Assessment Package (CalCAP) A series of continuous performance reaction time tests with large normative sample.
   Rationale: this is a more sensitive test of attention and concentration than tests previously used. (time = 10-15 minutes)

f) Controlled Oral Word Association (COWA) A word generation test.
Rationale: Krupp [24] showed differences on this test between controls and previously treated Lyme patients and it has several alternate forms. (time = 5 minutes)

g) Benton Visual Retention Test (BVRT)
Rationale: Halperin and Kaplan showed Lyme effects using this or a similar test (Wecksler) of memory. There are 3 alternate forms of the BVRT and only two of the Wecksler. (time = 15 minutes)

All test scores will be transformed into standard scores that will be calculated from published, age-corrected normative data. According to a previously described system, evidence of memory impairment is defined as scores that are 2 SD below the normative mean on any one of the tests of memory or more than 1 SD below the mean on two of the tests. A score of 70 or above on the Minnesota Multiphasic Personality Inventory is considered indicative of depression.

8. For patients whose persistent symptoms are of peripheral neuropathy including paresthesias or radicular pain, a detailed electromyographic examination of limb and paraspinal muscles will be performed with concentric needle electrodes. [25] Motor nerve and sensory-nerve conduction studies of the medial ulnar, peroneal, and tibial nerves will be performed with 10-mm surface recording and stimulating electrodes.

9. Testing for immune response to *B. burgdorferi* antigens in serum [26] and cerebrospinal fluid [27].

10. Cerebrospinal fluid and plasma specimens from patients enrolled in this study will be tested for the presence of *B.burgdorferi* DNA that encodes outer-surface protein (OspA). After extracting the DNA from the fluid, the samples are tested with a primer-probe set (called OspA 2,4) that targets a C-terminal portion of OspA (see

methods in the technical proposal). The samples are then amplified by 45 cycles of PCR. The amplicons are resolved by agarose gel electrophoresis and stained with ethidium bromide. After transfer to a nitrocellulose membrane, the membrane is hybridized with a P32-labelled DNA probe by southern blotting. The membrane is then exposed to autoradiography for 6-72 hr. Positive and negative controls are used throughout this procedure.

11. Culture. All CSF samples will be cultured for the presence of *B. burgdorferi* in BSK II medium and monitored by dark field microscopy for 6 weeks. Details of the methods are contained in [28].

12. For those women of child bearing age, urine pregnancy test will be performed. Menstrual cycle dates will be recorded in the patient diary.

13. Informed consent obtained in writing.

14. Serologic test for antibodies against *Babesia microti* [29].

15. Urine sample for subsequent testing of *B.burgdorferi* urine antigen [30].

B. Patient Number Assignment

After the investigator determines that the patient qualifies for the study, explains the nature of the study to the patient, and obtains informed consent documented in writing, the investigator or designee will assign each patient to the appropriate stratum based on the duration of their symptoms of chronic Lyme disease (less than four years, four to less than eight years, or eight to less than twelve years) and then assign a patient number for the study to the patient in numerical sequence within that stratum. The investigator/designee will record the patient's initials and date of administration of the first dose of the study medication on the case report form.

C. Medication Administration

The investigator will be given a blinded infusion bag or a pre-mixed 30 ml. syringe which contains the study drug required for the patient's first infusion. The treatments (ceftriaxone 2.0 grams in 100 ml. of 5% dextrose in water or placebo consisting of 100 ml. 5% dextrose in water with multi-vitamin to match the study medication for color) will be contained in identical looking infusion bags that will have patient numbers noted on the label and will be infused by gravity over 15 minutes. Syringes (containing either ceftriaxone 2.0 grams in 30 ml. of 5% dextrose in water or placebo consisting of 30 ml. 5% dextrose in water with multi-vitamin to match the study medication for color) will have patient numbers noted on the label and will be infused by I.V. push over 2-4 minutes. Approximate doses of multi-vitamin for color matching are:

	Infusion bag	Syringe
Ascorbic Acid	10 mg	3 mg
Vitamin A	200 mg	60 mg
Vitamin D	20 mg	6.0 mg
Thiamin	1.0 mg	0.3 mg
Riboflavin	0.8 mg	0.06 mg
Pyridoxine	0.3 mg	0.09 mg
Niacinamide	2.0 mg	0.6 mg
Vitamin E	0.01 I.U.	0.03 I.U.

All first infusions of study medication will be monitored by one of the physician/investigators. Subsequent infusions will be made under the supervision of the principal investigator or designee (study nurse). We expect that most of these infusions will take place in the patient's home. Following completion of the course of parenteral therapy patients will be dispensed oral medication containing doxycycline 100 mg. or identically appearing placebo (dextrose) which will be administered as one capsule every 12 hours for 60 consecutive days. Patients will be instructed that the oral medication may be taken with or without food.

Patient Instructions

All patients will receive instructions regarding possible side effects of treatment. Known side effects from ceftriaxone include but are not limited to: rash, diarrhea, GI upset, cholestasis, and anaphylaxis; known side effects from doxycycline include but are not limited to rash, photosensitization, diarrhea, GI upset, anaphylaxis, embryotoxicity and teratogenicity. Patients will also be instructed on signs of intravenous catheter infection. Patients will be able to reach the principal investigator or designee at any time to discuss any symptoms they may be experiencing during the administration of antibiotics or placebo.

E. Evaluations During Dosing Period

Ceftriaxone/Doxycycline or Placebo(Study Days 1 - 90).

1. Vital Signs Vital signs will be obtained on Days 1, 3, 5, 9,13,17, 21, 25 and 30.

2. Laboratory Tests Blood samples (15ml) will be obtained on Days 3, 5, 13, 21, 30, 45 and 75. CBC and liver function (as described in Section VI. A.4) will be performed on samples from days 5, 13, 21, 30, 45 and 75 for safety monitoring. Portions of these samples (from days 3, 5, 21 and 45) will be used for serial PCR testing to detect the appearance of borrelial DNA during treatment. Aliquots from these samples will also be stored at -70C as part of a specimen bank in patients with chronic Lyme disease for future studies. Urine samples (10 ml) will be collected on days 3, 5, 13, 21, 30, 45, and 75, and stored in aliquots at -70C. These specimens will be used for serial antigen testing and as part of the specimen bank for future studies of patients with chronic Lyme disease. No additional blood studies will be obtained from these patients except as noted below.

3. Compliance The patient will be queried at each visit regarding all medications taken, including all background medication. The information will be recorded in the Case Report Form.

A capsule count for the trial medication will be done at the day 45 and day 75 visit to monitor compliance. All unused medication should be returned to the Coordinating Center upon completion of the study. Any deviation from the regimen designated by the protocol must be explained; the patient should explain any apparent non-compliance. This will be recorded in the Case Report Form.

F. Post-Treatment Evaluations

Follow up evaluation by one of the physician investigators will occur at the completion of intravenous therapy (day 30) and then at the completion of oral therapy (day 90). Post-treatment evaluations will follow on days 180, and 360. At each of these visits all patients will repeat the SF™ 36 Health Survey and the Fibrositis [fibromyalgia] Impact Questionnaire. Physical examination with standardized recording of findings will also be recorded. Repeat neuropsychological testing to provide measures of immediate and delayed memory, conceptualization, copying, perceptual discrimination, depression and language will be performed at 90, 180 and 360 days. While all tests will be administered at the 90 day visit, only those tests which are not affected by "learning" will be repeated at 180 and 360 days (see methods for details). Blood (10cc) and urine will be collected at 90, 180 and 360 day follow up visits. Samples will be compared to baseline for an IgG response to borrelial antigens. In those patients in whom initial CSF is abnormal for any of the baseline parameters, follow-up lumbar puncture and CSF evaluations will be performed on day 90. Since patients will be enrolled over the first 3.0 years we will have the opportunity for long term follow-up. Therefore, we will also do telephone follow-up every 6 months of patients after the 360 day follow visit.

VII. MANAGEMENT OF INTERCURRENT EVENTS

A. Adverse Events

Throughout the duration of the study, the investigator will monitor each patient for evidence of drug intolerance and for the development of clinical and laboratory evidence of an adverse event. All adverse events, including those which have previously been reported as attributable to ceftriaxone, doxycycline or the presence of an intravenous catheter or which are judged by the investigator to be related to the therapy will be recorded on the case report form and followed to a satisfactory conclusion. The description of the adverse event will include the date, time of onset, duration, severity, etiology, relationship of the event to the study drug and any treatment or action required. The investigator will rate the severity of the adverse event according to the following definitions:

Severity:

1. Mild: The adverse event is transient and easily tolerated by patient.

2. Moderate: The adverse event causes the patient discomfort and interrupts the patient ' s usual activities.

3. Severe: The adverse event causes considerable interference with the patient's usual activities, and may be incapacitating or life threatening.

Likelihood that event is related to medication:

1. Concurrent Condition: An event, illness or effect of another drug not related to study drug (e.g., has not been reported for this drug, transient, or has no temporal relationship to study drug, or has a definite alternative etiology).

2. Remote ADE: An adverse event not commonly associated with this drug class, has little or no temporal relationship to the study drug, and a probable alternative etiology exists.

3. Possible ADE: An adverse event not commonly associated with this drug class, has a temporal relationship to the study drug, and a possible alternative etiology exists.

4. Probable ADE: An adverse event commonly associated with this drug class, has a temporal relationship to the study drug, lessened after the drug was discontinued, and no other etiology is apparent.

5. Definite ADE: An adverse event commonly associated with this drug class, has a temporal relationship to the study drug, reappeared on rechallenge, and no other etiology is apparent.

All deaths and serious adverse experiences which occur during the study or the post-therapy period, regardless of treatment group or relationship to drug, must be reported IMMEDIATELY BY TELEPHONE to the Clinical and Regulatory Affairs Branch (CRAB), DMID, NIAID. A serious adverse experience includes, but is not necessarily restricted to, events which are: [a] fatal; [b] life-threatening or potentially life-threatening; [c] permanently disabling; [d] an event requiring hospitalization; or [e] a congenital anomaly, cancer, or the result of overdose. It should be emphasized that, regardless of these criteria, any additional adverse experience which the investigator considers significant enough to merit immediate reporting should be so reported.

Every three months a report tabulating the adverse events by masked treatment assignment will be prepared by the statistical unit and transmitted in confidence to the to

Medical Officer, NIAID, and the DSMB Safety Officer. These reports will include all events and indicate for each whether or not they are considered attributable to the study medication.

B. Concomitant Drugs

If administration of any concurrent medication is necessary during the course of this study, dosage information, dates of administration, and indication for use must be reported on the appropriate case report form. Administration of additional antibiotics to treat intercurrent infectious diseases is acceptable provided the agent is not administered for longer that 7 days and/or is not active against *B. burgdorferi*. Administration of these agents must be documented on the case report form.

C. Premature Termination

All patients have the right to withdraw from the study at any time without prejudice. The investigator may discontinue any patient's participation when he feels it is necessary for any reason. Should a patient withdraw from the study, the reason must be documented on the case report form, and a final evaluation of the patient must be performed, as defined in Section VI.D.

D. Modification of Protocol

The investigators will not modify this protocol without first obtaining the concurrence of the project officer. The modification must be documented in writing. Any change in the protocol, except those necessary to remove an apparent immediate hazard to the subject, must be reviewed and approved by the Institutional Review Board prior to implementation. The investigators must submit protocol amendments to governmental agencies, and protocol modifications may be subject to Institutional Review Board review and approval.

E. Variation from Protocol for the Individual Patient

When significant variation from the protocol is deemed necessary for an individual patient, the investigator or other physician in attendance will inform the Project Officer. Any departure from the protocol will be authorized only for that one patient. A description of the departure from the protocol and the reason for it will be recorded on the appropriate case report form.

VIII. EVALUATION OF EFFICACY AND SAFETY

A. General Eligibility

The following conditions must be met if the patient is to be considered evaluable for the purposes of this study. The patient must have completed both baseline and follow up blood tests, CSF tests (where indicated), history and physical examinations and neuropsychiatric testing.

B. Evaluation of Antibiotic Therapy Efficacy

Success of ceftriaxone/doxycycline in the treatment of the symptoms of chronic Lyme disease will be determined by the investigator at the completion of the studies. The primary outcome for this study is defined as an improvement in the patients' health-related quality of life (HQL). Health-related quality of life will be measured using the SF-36 Health Survey. The SF-36 Health Survey includes eight multi-item scales that measure physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional and mental health. The eight multi-item scales are hypothesized to form two distinct higher-order factors, physical and mental, which provides the basis for scoring the SF-36 physical and mental health summary scales. Three additional multi-item scales from the medical outcomes study (MOS) will be used to measure cognitive functioning, pain, and role functioning but they will not be used for

the primary analysis of efficacy of antibiotic treatment. The SF-36 Health Survey (and the additional MOS measures) will be administered to study participants four times: at baseline, at one month (end of parenteral treatment), at three months (end of treatment), at six months and at one year..

To document the burden of Lyme disease on health-related quality of life, baseline scores for the SF-36 (and MOS scales) will be compared to general population norms. General population norms will be age adjusted to correct for differences in age between the study sample and the general population. Norm-based comparisons of SF-36 (and MOS scale scores) will also be conducted with 1, 3- and 6- month follow-up scores in both treated and placebo groups to evaluate change in the study participant HQL profile relative to general population norms.

To assess changes in HQL over time for study participants, a change score for each SF-36 (and MOS scale) will be calculated by subtracting the baseline score from the 1 month score, the 3 month score and the 6 month score. Using two standard errors of measurement (SEM), individual patients will be classified into 3 change categories: (1) those whose follow-up scores did not change more than would be expected by chance (“same group”); 2) those whose follow-up scores improved (more than 2 SEM's) more than would be expected by chance (“better group”); and 3) those whose follow-up scores declined (more than 2 SEM's) more than would be expected by chance (“worse group”). Based on prior studies with the SF-36, a 2 SEM change is 6.5 points for the SF-36 physical health summary scale and 7.9 points for the SF-36 mental health summary scale.

An overall determination of change in health status will be determined for each patient from the results of the two SF-36 summary scales (and the three MOS scales). Patients will be scored as improved if they are better on both scales or better on one scale and are not worse on the other scale.

The primary analysis will be an intent-to-treat analysis of all patients enrolled in the study. For secondary analysis, patients will be evaluated who have received at least 75% of the course of study therapy. Both analyses will involve comparisons between the proportion of patients improved at 6 months in the placebo and treated groups. A secondary analysis will compare the groups at the end of parenteral (30 days) and total active treatment (90 days). Comparison will consist of a χ^2 test at a significance level of 0.05, together with an estimate of treatment efficacy (risk ratio and risk difference) with 95% confidence intervals. As secondary and exploratory analyses, we will check to see if efficacy differs across subgroups by examining interaction terms in a multivariate analysis. We will also determine whether changes in patient performance on the supplementary MOS scales correlate with changes in the SF-36. Such subgroup analyses will need further confirmation in future studies. Nevertheless, their discovery may suggest important treatment interactions.

Tests will be done to ensure that no important potential confounders are unevenly distributed between patient groups. Regression analysis will be performed to determine the effect on results if any confounders are identified. An analysis stratified by duration of symptoms of chronic Lyme disease will be performed. An analysis stratified by site also will be performed to ensure that the results are not dependent on site. Among potential confounders and interaction variables are demographic variables (e.g., age, sex, race, education and location), medical history variables (e.g., previous psychiatric illness, prior history of arthralgia or arthritis, duration of symptoms, and interval between onset of acute Lyme disease and chronic symptoms) and physical examination variables (e.g., tender points and findings on neurologic examination).

C. Statistical Analysis

Study Sample Size

For a long course of high dose antibiotics to show efficacy there should be a substantial, durable difference between the antibiotic treated and the placebo groups. We shall assume that the natural healing rate is 0.40 or 40%. For an efficacy study in which the placebo rate is 0.4 and the expected treated rate is 0.65, we need a total of 124 (62 patients per group) to attain 80% power at a 0.05 (two-sided) level[31]. Dividing by 0.85 to allow for 15% dropouts gives a total of 146 patients. To achieve a 90% power, requires 194 patients with the same assumptions. The latter corresponds to an analysis with a 95% confidence interval half width of approximately 0.15. The sample size of 194 is also sufficient to allow us to rule out, at a one sided 0.025 level, a benefit of treatment as high as 0.25 in the healing rate, if the true healing rates are 0.4 for both the antibiotic treatment and placebo[32].

As an interim analysis it is appropriate to consider performing the study on seropositive patients with a sample size designed to show whether antibiotic treatment is equivalent to placebo treatment (a trial of equivalency). To determine the sample size necessary to show this, we need to define what "not effective treatment" means and to define the proportion of cases that would improve naturally without treatment or would improve by placebo treatment. We define treatment as ineffective if we can be reasonably sure (95%) that the difference between the healing rate under treatment (pE) and the natural healing rate (pS) is no greater than a defined magnitude (d). This is equivalent to showing that there is a high probability that the upper bound on a confidence interval for |pE - pS|) is less than d. (R. Makuch and R. Simon, Cancer Treatment Reports 62: 1037-1040, 1978). We shall assume that the natural healing rate is 0.10 or 10%. Then in order to have a minimum of 5 expected events (i.e. improvement) in the placebo group, we need a sample size of 50 in the placebo group. Assuming that the rate of improvement in the antibiotic treated group is the same as in the placebo treated group (pE = pS) and that there are 50 patients in each group, there is a 99% chance that we will be able to exclude any treatment difference greater than 25% using a 95% confidence interval (i.e., the power is 0.99 for d=0.25 and a=0.05). This sample size also gives 95% power for detecting a 20% treatment efficacy (d=0.20) and power = 0.80 for 15% (d=0.15). By

assuming a drop-out rate of 10% from each group a sample size of 55 patients in each group, or a total of 110 patients, would be required to be enrolled at the time of interim analysis.

Safety Evaluations

Safety will be assessed primarily by summarizing adverse event data. Adverse event incidence rates will be computed for each adverse event. The analysis of safety will be analyzed with Fisher's Exact Test. Laboratory data for each patient will be reviewed by the Data Safety Monitoring Board and the Medical Officer for clinically significant changes and will be summarized with descriptive statistics or other appropriate statistical methods.

Data Collection Procedures

Based on the extensive experience of the study Investigators in conducting clinical trials, a centralized data entry system has been selected to ensure the most effective approach. Data collection will be done at the study sites by a research nurse using multi-copy case report forms (CRFs). The study site will detach and retain a copy and send the remaining copies directly to the study coordinator at New England Medical Center. Upon receipt of the CRFs, each subject will be entered into the study log book and the CRF checked for completeness. When data are missing, an initial hand-written query will be generated, a copy of the form retained, and the incomplete form returned to the study site research nurses. Data from the originally complete CRFs and returned completed CRFs will be entered into a computer database, using a program with built-in checks for ranges and limits of values and logic checks. A computerized query will then be generated on each CRF and will be sent back to the study site hospital nurse for any needed completion and verification. The answers to these queries will in turn be sent back to the Study Coordinator where all data from the CRF including new information will then be entered into the computerized database for a second time. The program will once again run the

data checks for additional problems. There, following checking, they will be combined with the data from the CRFs. A SAS database will be created and analyses will be done on microcomputers on the NEMC IBM mainframe computer. The initial and follow-up data to be collected on all patients is described above.

IX. CASE REPORT FORMS

The investigators will review the case report forms for completeness and accuracy, and will sign and date the forms where indicated. The investigators and the Data Safety Monitoring Board will meet to review all completed case report forms for completeness and legibility.

All records which support case reports of this study must be retained in the files of the principal investigator.

X. INFORMED CONSENT

Each subject must voluntarily sign the informed consent form before participating in this study. Obligation for obtaining the informed consents for both studies of each subject is assumed by the investigator. The study protocols and informed consent forms must be approved by the investigator's IRB. Each original will be retained by the investigator and a copy will be given to each patient.

XI. PATIENT CONFIDENTIALITY

All reports and communications relating to patients in the study will identify each subject only by the patient's initials and by the patient's study number. The investigator agrees to retain complete patient identification on the confidential patient follow-up form, which will be used for the purpose of long-term follow-up if needed. This information will be treated with strict adherence to professional standards of confidentiality.

Case report forms will be reviewed for completeness and acceptability by the principal investigator or designee at the study site. Portions of the patients medical records pertinent to the study will be reviewed by these personnel and possibly by FDA personnel to assure accuracy.

XII. USE OF INFORMATION AND PUBLICATION

To allow for the use of the information derived from this clinical study and to insure complete and thorough analysis, the investigator is obligated to provide the NIAID with complete test results and all data developed in this study. Included in the data will be results of testing for concordance for seropositivity by a second laboratory. Should the investigator choose to publish the results of this study, a copy of the manuscript will be provided to NIAID at least 30 days prior to the date of submission to the intended publisher.

XIII. COMPLETION OF STUDY

The investigator will complete and report this study in satisfactory compliance with the protocol within 12 months of final data collection.

If, at the completion of the study, the protocol treatment is found to be effective, patients who have received the placebo will be offered the study treatment regimen (30 days of intravenous Rocephin, 2 grams per day, followed by 60 days of oral doxycycline, 200mg per day) through their regular health care insurance coverage. If coverage for treatment is denied by the patient's regular health care insurance, or if a patient does not have health insurance, the costs of such treatment will be paid through the contract supporting these studies.

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