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A Placebo-controlled Trial of Folate with B12 in Patients with Schizophrenia with Residual Symptoms in Ethiopia Using a Sequential Parallel Comparison Design

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Authors' contributions

Authors DCH, TS, AF, DB, ST and CPCB conceived the trial question and have been involved in all stages of the study design. These individuals also participated in writing the grant and study protocol, submitting to the funding body, and applying to the ethics committee. They are responsible for trial management, staff training and supervision. Authors CEO, SB and AM are clinical research coordinators involved in developing and operationalizing the trial procedures at the study site. Author DAS is the statistician who developed the study design. All authors have read and approved the final manuscript.

Study Protocols

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ABSTRACT

Background: Approximately 30% of patients with schizophrenia suffer from treatment-resistant psychotic symptoms, which can produce substantial distress, result in

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hospitalization and disrupt school or work functioning. Studies have found low blood folate concentrations in psychiatric populations and recent reports have consistently linked schizophrenia to low folate levels. We aim to examine the efficacy of a four-month trial of folate with B12 supplementation for reducing symptoms of schizophrenia.
Methods: This study is a randomized, sequential parallel comparison design (SPCD) for double-blind phase fixed dose, 4-month trial of folate plus B12 as add-on therapy to reduce symptoms of schizophrenia. Participants will be adults (ages 18 to 65 years) diagnosed with schizophrenia, any subtype, who are psychiatrically and medically stable, but have residual positive or negative symptoms of moderate or greater intensity, despite antipsychotic treatment. The study is divided into 2 double-blind phases of 56 days each. Two hundred total participants will be randomized to adjunctive treatment with either folate with vitamin B12 (n=50) or placebo (n=150), with a 2:3:3 ratio for random assignment to the treatment sequences drug/drug (DD; n=50), placebo/placebo (PP; n=75), and placebo/drug (PD; n=75), while all continue to receive their current antipsychotic agent for the duration of the study. Diagnosis will be established using the Structured Clinical Interview for DSM-IV for clinical trials (SCID-CT). The primary outcome measure will be change in symptom severity measured by the change from baseline in Positive and Negative Syndrome Scale (PANSS) total score. Secondary outcome measures will include change in severity of psychotic symptoms as measured by the PANSS psychosis subscale score; and change in severity of negative symptoms as measured by the modified Scale for Assessment of Negative Symptoms (SANS) total score. Key assessments for primary and secondary outcomes will be conducted at baseline, week 8, and week 16.
Trial Registration: Clinicaltrials.gov identifier: NCT01724476.

Keywords: Folate; vitamin B12; schizophrenia; negative symptoms; sequential parallel comparison design; Ethiopia.

ABBREVIATIONS

SPCD: Sequential Parallel Comparison Design; MTHFR: methylenetetrahydrofolate reductase; PANSS: Positive and Negative Syndrome Scale; SANS: Scale for the Assessment of Negative Symptoms; CGI: Clinical Global Impression Scale; SF-36: Modified Medical Outcomes Study Short Form-36;SEQ: Side Effect Questionnaire; C-SSRS: Columbia Suicide Severity Rating Scale; AIMS: Abnormal Involuntary Movements Scale; SAS: Simpson Angus Scale for Extrapyramidal Symptoms; BAS: Barnes Akathisia Scale; EPHI: Ethiopian Public Health Institute.

1. INTRODUCTION

About 30% of patients with schizophrenia suffer from treatment-resistant psychotic symptoms, which can produce substantial distress, result in hospitalization and disrupt attempts to function in school or work. In addition, negative symptoms often remain largely treatment refractory and are a major contributor to disability in people with schizophrenia. Vitamin supplementation with folate and B12 represents a safe and inexpensive approach, which could significantly improve outcomes for patients with residual symptoms.

Folate and vitamin B12 deficiencies occur primarily as a result of insufficient dietary intake [1]. Folate is present in high concentrations in legumes, leafy green vegetables, and some

fruits; so lower intakes can be expected where the staple diet consists of unfortified wheat, maize, or rice, and when the intake of legumes and folate-rich vegetables and fruits is low. There are few data on folate intakes in different locations, but one would anticipate that status is poorer in populations who rely on unfortified wheat or rice as a staple, and consume low amounts of legumes and green leafy vegetables [1]. Consequently, micronutrient intakes are often inadequate [2]. Hence, coexisting deficiencies of iron, zinc, vitamin A, vitamin B-12, and folate have often been reported [3]. Fortification of grains with folic acid has increased folate intake in several developed countries [4] but these foods are generally not available in Ethiopia.

In Ethiopia, protein and nutrient deficiencies are common and malnutrition is a concern [5, 6]. To date, studies of nutrient deficiencies have been conducted only in select regions of this country. One study of women's nutritional status in Ethiopia found that of the 970 women participating in this study, nearly half of the sample (46.1%) had severe folate deficiency and 21.2% of the sample had marginal folate deficiency, while only 32.7% had optimal levels of serum folate [7]. All regions were significantly affected with folate deficiency except the Dire-Dawa, Tigray and Afar regions [7]. Nonetheless, another study confirmed that the Addis Ababa, Amhara and southern Ethiopia regions have among the lowest prevalence of folate deficiency within the country, yet even in these regions the prevalence of folate deficiency is substantial with only 58% or women achieving optimal folate levels in Addis Ababa, 42% in Amhara and 26% in southern Ethiopia. Thus, folate deficiency is clearly a concern throughout Ethiopia and related to diet [7].

1.1 Folate

Folates are in the diet as a complex mixture of polyglutamate conjugated compounds (folypoly-glutamates) that are deconjugated in the cells of the intestinal wall to produce the monoglutamate form that is more readily absorbed by active membrane transport. The availability of folate in the typical Ethiopian diet is unclear, though general nutrient deficiencies and malnutrition are recognized concerns in the country. The concentration of folate in serum is considered an indicator of short-term folate status, whereas folate concentration in red blood cells is considered a good index of longer-term body folate stores [8]. Folate is converted to 5-methyltetrahydrofolate (5-MTHF) by methylenetetrahydrofolate reductase (MTHFR); 5-MTHF is the active form, which serves as a methyl (single carbon) donor for a wide range of critical metabolic pathways. 5-MTHF combines with B12 to form a chemical structure that is required for the synthesis of methionine from homocysteine. Methionine in turn is required for the synthesis of S-adenosylmethionine (SAM) which is the methyl donor for many biochemical reactions that influence brain development and function, including the synthesis of DNA and RNA, methylation of DNA (which regulates access to transcription factors) and synthesis of amino acids and monamines, including neurotransmitters implicated in negative and positive symptoms of schizophrenia—glycine, serine, dopamine and serotonin. As an indication of the wide range of biological activity, hypofolatemia has been linked to neural tube defects, Down's syndrome [9] autism [10], depression [11], Alzheimer's disease [12], cognitive impairment in the elderly [13], cardiac disease [14], cancer [15], and schizophrenia [16,17]. The only reported risk of folate supplementation is a potential masking of anemia related to low B12 levels. Subjects enrolled in this study will be screened at screening and week 8 for megaloblastic anemia, which is an exclusionary criteria. If a subject's complete blood count (CBC) at week 8 is suggestive of megaloblastic anemia they will be withdrawn from the study and referred for appropriate medical treatment.

1.2 Cobalamin (B12)

Cobalamin is bound to protein in the diet and requires gastric hydrochloric acid and intrinsic factor for release and absorption from the gut. B12 serves as a co-factor with 5-MTHF in the conversion of homocysteine to methionine, which results in the production of a methyl group used for methylation reactions in several pathways, including synthesis of neurotransmitters and DNA. B12 deficiency can result from disorders of gastric absorption (celiac disease, Crohn's disease, surgical gastric resection), dietary insufficiency (including vegetarian diets) or from medications that interfere with B12 absorption (proton pump inhibitors, H2 receptor antagonists or metformin). B12 deficiency most commonly presents with cognitive impairment, but can also result in anemia, fatigue, paresthesias, or depression. No upper limit has been defined for B12 supplementation since no adverse effects of B12 have been identified.

1.3 Preliminary Folate Studies in Schizophrenia

Over 20 studies have found low blood folate concentrations in psychiatric populations [18,19]. Recent studies have consistently linked schizophrenia to low folate levels, whereas a link to elevated homocysteine has been inconsistent. Koren and colleagues [20] found that serum folate concentrations were significantly reduced in 70 schizophrenia patients compared to control subjects sampled from a general hospital. Godfrey and colleagues [21] found that 33% of 123 patients with either schizophrenia or depression had folate deficiency (red-cell folate below 200g/l), and that both groups of patients exhibited significant clinical improvement and enhanced "social recovery" with a six-month course of methylfolate 15 mg daily compared to placebo. In a sample of 53 schizophrenia outpatients, Herran and colleagues [22] found low serum folate levels that correlated negatively with scores on the Clinical Global Impression Scale and the total score on the PANSS. In a three-month, placebo-controlled cross-over trial of folate 2mg/d plus B12 400g/d in 42 treatment-resistant schizophrenia patients with elevated homocysteine levels, Levine and colleagues [23] demonstrated a significant ($p=0.01$) 20% reduction in PANSS total score which displayed a steady slope for the entire 3 month trial and persistence of effect over 3 month follow-up. Two placebo-controlled trials of folate supplementation have reported therapeutic benefit; in one, methylfolate improved symptoms and level of functioning in 17 patients with schizophrenia [21] whereas folate plus B12 supplementation improved psychotic symptoms in a second trial [23], thereby suggesting that B12 may enhance folate supplementation in its role as a co-factor in critical pathways. Roffman and colleagues found that adjunctive folate plus B12 significantly improved negative symptoms in 140 patients with schizophrenia as measured by change in SANS total score [24]. Furthermore, Roffman and colleagues also investigated potential influence of specific genetic vulnerabilities upon the relationship between negative symptoms and folate levels in this population. Four genetic variants, MTHFR 677T, MTR 2756A, FOLH1 484C, and COMT 675A were found to be significant predictors of negative symptom severity [25]. We recently found associations between low dietary intake, low serum folate concentrations and negative symptoms [16,26] in schizophrenia patients, as well as between MTHFR C677T status and negative symptoms [27] and cognitive deficits [27]. Low folate levels attributable to anticonvulsant medication [28,29] or dietary deficiency [30] have been linked to apathy, psychosis and cognitive impairment, and in depressed patients has been associated with apathy and poor response to pharmacotherapy [31,32].

Although the evidence for a relationship between blood folate concentrations, MTHFR status and schizophrenia is strong, it remains unclear if folate supplementation will improve negative or psychotic symptoms, and whether B12 will enhance the therapeutic effect. Additionally, there is little data on folate supplementation in schizophrenia for developing countries such as Ethiopia. Food products in Ethiopia are not supplemented with folate, which suggests that the effects of folate supplementation may be even more pronounced. The nutrient deficiency and malnutrition experienced by the Ethiopian population, in addition to the high rates of folate deficiency found in preliminary studies, suggests that folate with vitamin B12 supplementation could represent a safe and inexpensive approach that could significantly improve outcomes for patients with schizophrenia with residual symptoms in Ethiopia. The current study, with its pioneering study design, will be a significant step towards understanding the role of folate supplementation in regulating the symptoms of schizophrenia, specific to a folate-deficient population.

The primary aim of the trial is:

1. To examine the efficacy of a four-month trial of folate supplementation with B12 for reducing symptoms of schizophrenia as measured by the change from baseline in Positive and Negative Syndrome Scale (PANSS) total score.

The secondary aims of the trial are:

1. To examine the efficacy of folate supplementation with B12 for psychotic symptoms as measured by the PANSS psychosis subscale score.
2. To examine the efficacy of folate supplementation with B12 for negative symptoms as measured by the modified Scale for Assessment of Negative Symptoms (SANS) total score.
3. To examine the relationship among response of PANSS total score, negative and positive symptoms and baseline serum and plasma folate, RBC folate, homocysteine and B12 concentrations, tobacco intake, and MTHFR C677T status.
4. To examine the relationship between response of negative and positive symptoms and the change in folate concentrations, RBC folate, and change in plasma homocysteine concentrations.

We hypothesize that persons with schizophrenia with residual symptoms who are given folate with B12 supplementation will show greater reduction of negative and/or psychotic symptoms of schizophrenia and greater reduction of serum homocysteine concentration compared with those taking standard antipsychotic medication alone.

2. METHODOLOGY

2.1 Research Design

The study will be a randomized, Sequential Parallel Comparison Design for double-blind phase, fixed dose, 4-month trial of folate plus B12 or placebo as add-on therapy among subjects with schizophrenia, any subtype, with stable, residual positive or negative symptoms. The Sequential Parallel Comparison Design (SPCD) paradigm was developed by Dr. David Schoenfeld and Dr. Maritzio Fava of Massachusetts General Hospital (MGH) in 2003. The SPCD paradigm was designed in order to address the issue of high placebo response rate in clinical trials, particularly in the field of psychiatry where treatment response

and efficacy often relies on somewhat subjective clinical interviews and assessments. Unlike a conventionally designed single phase trial, SPCD utilizes two sequential, double-blind phases, which enables one to account for placebo non-responders during efficacy analyses. Participants in an SPCD trial will be randomized to one of three treatment sequences: 1) drug during Phase 1, drug during Phase 2 (Drug/Drug sequence), 2) placebo during Phase 1, placebo during Phase 2 (Placebo/Placebo sequence), or 3) placebo during Phase 1, drug during Phase 2 (Placebo/Drug sequence). While all subjects from Phase 1 will typically enter Phase 2, only placebo non-responders during Phase 1 will be included in the Phase 2 efficacy analysis thereby allowing researchers to statistically control for the detrimental effect of high placebo response. In the current trial, the signal detection of a drug-placebo difference will be enhanced in the second phase of the study, where only non-responders to placebo in the first phase will be included in the analyses of the second phase (thereby including only a sample of prospectively-defined placebo non-responders).

A further benefit of the SPCD paradigm is its ability to achieve an equivalent power as in conventional, single-phase trials, while utilizing a smaller sample size. An SPCD trial with a sample size of *n* will achieve a power that is 10-25% higher than a conventional single-phase trial with the same *n*. Alternatively, if a specific power is specified, an SPCD paradigm will require 20-50% fewer participants to achieve that power as compared with the number of subjects that would be required in a single-phase trial. This design is particularly valuable in global clinical trials such as the current trial, as it provides the greatest power to detect an effect of the intervention within the limits of the sample sizes that can be realistically achieved through the collaborative and funding limitations[33]. In the current trial, the efficiency of the sequential parallel comparison design will allow us to reduce the sample size requirements by 50%. Preliminary studies in psychiatric populations have demonstrated these various benefits of utilizing the SPCD design. Boessen et al. [34] compared the sample size requirements of a 8-week randomized controlled trial (RCT) scenario to sequential parallel comparison design scenarios of equivalent length or longer. For the comparative scenarios utilizing antidepressant clinical trial data, the 8-week SPCD scenarios required 50-53% fewer participants than the 8-week RCT scenario.

In accordance with the sequential parallel design, the 112-day, double-blind adjunctive treatment with folate and vitamin B12 is divided into two phases of approximately 56 days each, with assessments performed every 14 to 28 days. During the first phase of double-blind adjunctive treatment, eligible patients (n=200) are randomized to *adjunctive* treatment with folate plus vitamin B12 or placebo, with a 2:3:3 ratio for random assignment to the treatment sequences drug/drug (n=50), placebo/placebo (n=75), and placebo/drug (n=75). Assuming a 10% drop-out rate during the first phase, 136 patients on placebo will complete the first 56-day phase, and 45 patients on folate and vitamin B12 will complete the first 56-day phase (See Fig. 1 Schematic Diagram of Study Flow).

Patients randomly assigned to the drug/drug sequence remain on the same dose of folate and vitamin B12 during the second phase, regardless of whether patients have or have not responded during the first phase. For those randomly assigned to the placebo/placebo sequence, both responders and non-responders to placebo (n=75) during the first phase will remain on placebo during the second phase. For those randomly assigned to the placebo/drug sequence, both responders and non-responders (n=75) to placebo during the first phase will go on to receive folate and vitamin B12 during the second phase.

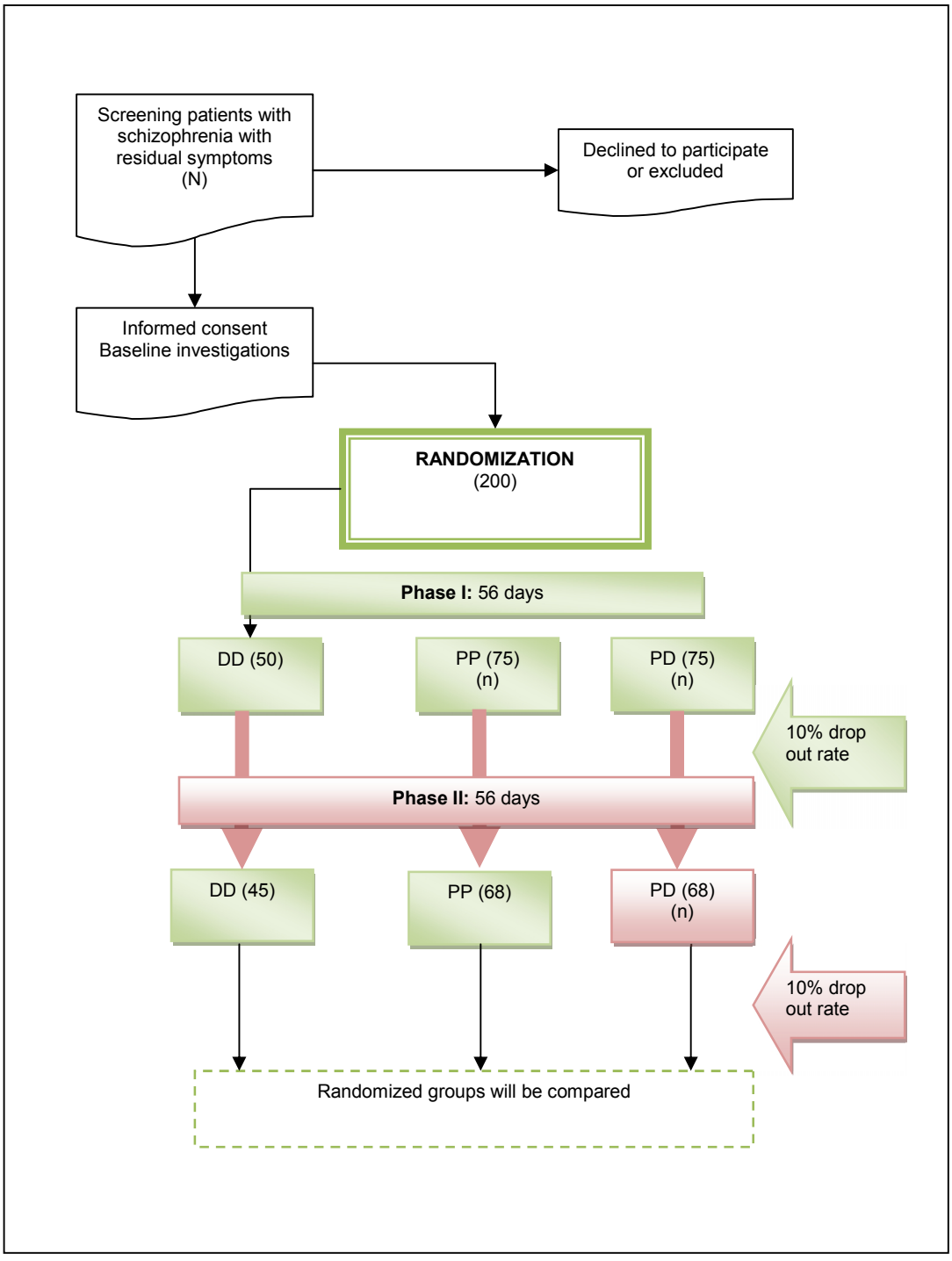

Fig. 1. Schematic diagram of study flow

Diagram displays the flow of study participants from initial screening; through randomization to drug/drug treatment sequence, placebo/placebo treatment sequence, and placebo/drug treatment sequence; to study completion

The pre-randomization to the drug-drug, placebo-placebo, or placebo-drug sequences is designed to decrease placebo-response and to facilitate a smooth transition between phases. The overall odds of receiving placebo or folate plus vitamin B12 at any point during the study will be reflected in the consent form, which will not mention two phases to minimize the risk of a change in treatment outcome expectations at the beginning of the second phase.

2.2 Research Setting and Participants

The study will be conducted at Amanuel Psychiatric Hospital. This hospital has a 360 bed capacity in-patient unit and has remained to be the only psychiatric referral hospital for the whole nation for decades. On average about 400 patients attend the outpatient clinic daily, 39% of which are patients suffering from schizophrenia, and most of whom receive conventional antipsychotic medications.

Approximately 240 consecutive outpatients with schizophrenia from Amanuel Psychiatric Hospital will be enrolled and screened for eligibility. The goal is to randomize and complete 200 subjects. Inclusionary and exclusionary criteria are designed to identify a sample of psychiatrically and medically stable patients with schizophrenia, any subtype, with residual positive or negative symptoms of moderate or greater intensity, despite antipsychotic treatment.

The inclusion criteria for this study are: male or female; age 18-65 inclusive; a clinical diagnosis of schizophrenia, any subtype; treated with an antipsychotic medication for at least 6 months and at a stable dose for at least 6 weeks; and a Positive and Negative Syndrome Scale (PANSS) total score of at least 60, with at least a 3 (moderate) on one negative symptom item or on one positive symptom item. Exclusion criteria are: inability to provide informed consent or do not have a guardian to consent; serum B12 concentration less than 300ng/L and CBC results suggestive of megaloblastic anemia in the clinical judgment of the investigator; current use of folate or B12 or other multivitamin with B12 supplementation; current use of any of the following medications: phenobarbital, phenytoin, carbamazepine, valproic acid, fosphenytoin, primidone or pyrimethamine; antimalarial drugs; current history of alcohol abuse or dependence or other substance abuse (nicotine allowed); unstable medical illness; unstable psychiatric illness; seizure disorder; pregnancy or nursing; and known allergy to folate, vitamin B12, multivitamin, or B-complex vitamin.

2.3 Sample Size

The Sequential Parallel Comparison Design (SPCD) provides the greatest power to detect an effect of folate and vitamin B12 within the limits of the sample sizes that can be realistically achieved through the collaborative and funding limitations[33]. In fact, the signal detection of a drug-placebo difference is enhanced in the second phase of the study, where only non-responders to placebo in the first phase are included in the analyses of the second phase (thereby including only a sample of prospectively-defined placebo non-responders). Therefore, the efficiency of the sequential parallel comparison design will allow us to reduce the sample size requirements by 50%. The required sample size, after considering an 11% contingency would be 200.

2.4 Intervention Schedule

All participants will be maintained on their pre-study antipsychotic medication and usual treatment will continue under the treating physician. All medication changes made by the participant's clinical team will be recorded throughout the study. The active intervention, folate with B12, will be given at a dose of 2mg folate with 400µg B12 per day. The comparison group will receive an inactive placebo compound of identical color, texture and taste. The treatments will be given for the full 16 week duration of the study. Trial medication will be dispensed every four weeks: baseline, and weeks 4, 8 and 12. Medication compliance will be assessed at weeks 4, 8, 12, and 16. To assess compliance, participants will be instructed to return all empty medication bottles and any left-over tablets to the study staff at each follow-up visit coinciding with medication resupply.

2.5 Outcome Measures

The primary outcome measures for this study will be measured by the change from baseline in Positive and Negative Syndrome Scale (PANSS) total score. The following clinical rating scales will comprise the clinical efficacy assessment battery: 1) Positive and Negative Syndrome Scale (PANSS) [35]: includes three primary symptom subscales (positive symptoms, negative symptoms, and general psychopathology); 2) Scale for Assessment of Negative Symptoms (SANS) [36]: which is more sensitive than the PANSS negative symptom subscale to assess negative symptom changes over time; 3) the Clinical Global Impression Scale (CGI) [37]: a three-item scale that assesses treatment response in psychiatric patients. Categories include severity of illness, global improvement, and efficacy index; the goal is to provide a summary score of the person's overall functioning; 4) The Modified Medical Outcomes Study Short Form-36 (SF-36) will be used to measure general health status and functioning in six domains[38]; and 5) the LIFE-Satisfaction item will be used to assess quality of life through overall satisfaction rating. This item is intended to convey the subject's contentment with the various areas of functioning in his/her life, and not his/her actual level of functioning. This includes the gratification received from these activities and the degree to which the subject thinks his/her needs and desires are fulfilled. Subject's average level of satisfaction will be rated on a 5 point Likert scale ranging from 1 (very good) to 5 (very poor).

For the nutrition assessments, subjects will complete a 24-hour food intake recall to assess their dietary intake. Food records will be reviewed for completeness and analyzed by the Ethiopian Public Health Institute (EPHI). However, in addition to the 24-hour food intake recall, the Ethiopian Food Habit Questionnaire will be also completed, which is an assessment designed to capture Ethiopian-specific dietary intake and habits.

Medical evaluation will be centered on a comprehensive battery of laboratory assays, which will be performed both as a participant safety measure, and to investigate primary and secondary outcome measures by examining the relationship between response of negative and positive symptoms and the change in folate, RBC folate, and plasma homocysteine concentrations, as well. Laboratory assays will include standard screening blood tests, including Complete Blood Count (CBC) with differential, electrolytes, creatinine, BUN, glucose, liver enzymes, calcium, phosphate, magnesium, albumin, lipid profile (total cholesterol, triglycerides, HDL and LDL), and inflammatory markers (IL-2, IL-1B, IL-6, IL-8, IL-10, IL-12p70, TNF, C-reactive protein, GM-CSF). In addition, blood will be obtained for assay of serum B12, folate concentrations, RBC folate, plasma homocysteine concentration,

and DNA. Additional medical evaluations will include the collection of vital signs (weight, and standing and supine pulse and blood pressure) and consumptive habits (nicotine use, alcohol use, caffeine use, and khat use), which will be assessed at every visit.

2.5.1 Safety assessments

Safety assessments will be conducted at every study visit to document the presence of any spontaneously reported side effect or adverse events. The Side Effect Questionnaire (SEQ) and Side Effect Questionnaire–Abbreviated (SEQabrv) will be the primary instruments used to detect side effects. The SEQ was developed to capture the presence and severity of possible side effects specific to the study medication. The SEQabrv is an open ended inquiry about "any physical or health problems experienced during the past 2 weeks," and the severity of those physical or health problems. Subjects are encouraged to contact the principal investigator or his designee at any time between visits concerning adverse events or worsening of symptoms. Suicidal ideation is assessed at each visit through the Columbia Suicide Severity Rating Scale (C-SSRS) [39]. Subjects who are felt by the rater or study psychiatrist to be at high risk for suicide will be discontinued from the study and referred for hospitalization and further treatment as clinically indicated. The Abnormal Involuntary Movements Scale (AIMS)[40], Simpson Angus Scale for Extrapyramidal Symptoms (SAS) [41], and Barnes Akathisia Scale (BAS) [42] will be used to assess extrapyramidal side effects.

2.5.2 Rater training and inter-rater reliability

All ratings will be performed by a team of three raters who have extensive experience with all clinical rating scales used in this study. Additional training for raters will be conducted by Addis and MGH investigators. Inter-rater reliability will be re-established before the start of this project and will be repeated every three months by use of taped interviews. When assessing inter-rater reliability, the participant's responses are independently and simultaneously rated by two observers. The assessments will be conducted by two research psychiatrists and Cohen's kappa [43] will be calculated to show the degree of agreement. In our training and monitoring sessions, any difference of greater than one point results in a careful review of the item with both members of the team. Additionally, in order to conduct a reliability analysis on the stability of the PANSS and the SANS, every consented individual will complete the measures within approximately 2 weeks and prior to receiving study drug [44]. First, estimates of internal consistency using Cronbach's alpha will be calculated. This method will provide an indication of the interrelatedness of the items. Second, Pearson correlations will be calculated on the responses from those participants who completed the questionnaire on two occasions to examine the scale's test-retest reliability.

2.6 Assessment Schedule

The study will be comprised of eight study visits total with the full battery of assessments conducted at three key time points: baseline (before the initiation of trial medication), week 8, and week 16 (end of intervention).

2.6.1 Screening assessments

All assessment scales, including the consent form and any recruitment materials will be developed in English, translated to the local language and then translated back to English to verify consistency.

Informed consent will be obtained after a diagnosis of schizophrenia, any subtype, is determined by the SCID-CT, which will be completed by a research psychiatrist/clinician using all available clinical data. A medical and psychiatric history and physical examination will be obtained by a physician or research nurse to assess inclusionary and exclusionary criteria, including current medications and an assessment of Consumptive Habits (caffeine, tobacco, alcohol, and substance use). A trial physician will administer the PANSS and SANS to determine whether symptom severity inclusionary criteria are met. The C-SSRS will be administered by a site study psychiatrist to assess suicidality. A fasting blood sample will be obtained to perform the full battery of laboratory assessments and a pregnancy test will be done for women of child bearing potential. All pregnancy tests must be negative for a female patient to continue participating in the study. Vital signs will be obtained at the screening visit, which will include height (screening only), weight, blood pressure (standing and sitting), and pulse (standing and sitting).

2.6.2 Baseline assessments

The baseline visit will be comprised of the clinical efficacy assessment battery (PANSS, SANS, CGI, SF-36, and LIFE-Satisfaction item), nutrition assessments (24-hour food intake recall and Ethiopian Food Habits Questionnaire), medical evaluations (laboratory assays, vital signs and consumptive habits), and safety assessments (SEQ, SEQabrv, C-SSRS, AIMS, SAS, BAS). After the completion of all screening and baseline assessments, subjects will be randomized, double-blind, to receive adjunctive folate 2mg and B12 400 µg/day (n=50) or placebo (n=150), with a 2:3:3 ratio for random assignment to the treatment sequences drug/drug (n=50), placebo/placebo (n=75), and placebo/drug (n=75).

2.6.3 Follow-up assessments

Subjects will meet with a research assistant or another member of the research team in the hospital at weeks 2, 4, 8, 10, 12, and 16. Weeks 2 and 10 study visits will consist of vital signs, the C-SSRS, Consumptive Habits assessment and the SEQabrv. Weeks 4 and 12 study visits will consist of the PANSS, SANS, LIFE-Satisfaction item, SEQabrv, C-SSRS, vital signs, Consumptive Habits assessment. Weeks 8 and 16 study visits will include all baseline clinical, nutritional, medical, and safety assessments. In addition, during weeks 8 and 16 a fasting blood draw and repeat pregnancy test will be completed. Subjects will take the study medication during the entire 4 months (16 weeks) of study participation. Study drug will be dispensed at baseline, weeks 4, 8 and 12. Medication compliance will be assessed at weeks 4, 8, 12, and 16.

2.7 Statistical Analysis

2.7.1 Power analysis and primary aim analyses

Data will be examined prior to analysis using descriptive statistics. Randomized groups will be compared on continuous variables using ANOVA and on categorical predictors using chi-square tests. Continuous measures will be assessed for normality and transformations will be applied as necessary. Our primary analyses will be intent-to-treat using all available data on all subjects. If a subject drops out in phase 1, then the last observation will be carried forward in phase 1, while this subject's data will not be used in calculating the part of test statistic corresponding to phase 2. If a subject drops out in phase 2, then last observation will be carried forward in phase 2 and all data from this subject will be used in the analyses. Sensitivity analyses will include analyses on completers only and analyses using missing

values on the response variable replaced by predicted values from linear or generalized linear models of the longitudinal data over time. Subjects will be randomized to one of three treatment sequences: 1) drug during Phase 1, drug during Phase 2 (Drug/Drug sequence), 2) placebo during Phase 1, placebo during Phase 2 (Placebo/Placebo sequence), or 3) placebo during Phase 1, drug during Phase 2 (Placebo/Drug sequence). Based on this design we will run 3 sets of analyses. We assumed the following drop-out rates: 10% in stage one and 10% in stage 2. The probability of seeing a significant result at a two sided p=0.05 level will be 86%, under the following assumptions: The probability of response for the treated group will be 50% and the probability of response in the placebo group will be 35% in the first phase of the study and the probability of response in the treated group will be 30% and the probability of response in the placebo group will be 10% in the second phase where placebo non-responders are randomized between drug and placebo.

3. DISCUSSION

As evidence for the potential role that blood folate concentrations may play in schizophrenia continues to grow, folate supplementation has become an important area of research within this psychiatric population. Its availability, low cost, and low side effect profile make folate supplementation an attractive area of potential intervention, particularly in low and middle income countries such as Ethiopia where vitamins and supplements are more easily accessible than drug interventions, and their low side effect profile do not necessitate close medical monitoring, which may not be consistently available to this patient population in developing countries.

Though to date no studies on folate supplementation in schizophrenia have been conducted in Ethiopia, preliminary studies have found high rates of folate deficiency across the country and pervasive malnutrition is linked to general nutrient deficiency. Furthermore, the fact that the government of Ethiopia does not supplement folate in its food products suggests that the effects of folate supplementation may be even more pronounced in this population. For all these reasons, folate with vitamin B12 supplementation could represent a safe and inexpensive approach that may significantly improve outcomes for patients with schizophrenia with residual symptoms in Ethiopia. Participants will begin to enroll in the trial in July 2014 and recruitment will continue for approximately 12 months.

4. CONCLUSION

The significance of the current study is further bolstered by the use of the Sequential Parallel Comparison Design (SPCD) which allows a trial to utilize a smaller sample size while maintaining equivalent power to a conventional single-phase trial. This benefit of SPCD is particularly valuable when conducting global clinical trials in developing countries where large sample sizes may be difficult to realistically recruit and manage. The efficiency of the sequential parallel comparison design will allow us to reduce the sample size requirements in this trial by 50%. The current study represents a significant step towards understanding the role of folate supplementation in regulating the symptoms of schizophrenia, specific to a folate-deficient population.

CONSENT

Patients will begin to enroll in this trial in June 2014 and recruitment will continue for approximately 12 months. All authors declare that written informed consent will be obtained from the patients before any study procedures are performed.

ETHICAL APPROVAL

The study has been reviewed and approved by both the Massachusetts General Hospital Institutional Review Board (Protocol #2011-P-002667) and the Addis Ababa University College of Health Science Institutional Review Board (Protocol #040/12/SPH). All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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