Zinc Supplementation and Cardiovascular Risk in HIV

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Pilot Study of Zinc Supplementation and Cardiovascular risk in HIV

Principle Investigator:

Grace McComsey, MD, FIDSA
Professor of Pediatrics and Medicine
Director, Dahms Clinical Research Unit
Chief, Pediatric Infectious Diseases & Rheumatology

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Study Team Roster:

Site Investigator: Grace McComsey, MD| 11100 Euclid Ave Cleveland Ohio 44106| Phone: 216-844-2739|Fax: 216-201-4607

Research Staff: 11100 Euclid Ave Cleveland Ohio 44106| Phone: 216-844-2739|Fax: 216-201-4607|All CITI trained, CREC certified and IRB trained to consent study participants

Danielle Labbato, BSN
Lisa Pirro, BSN
Margaret Meek
Caitlin Hearn

Participating Study Site: University Hospitals Cleveland Medical Center| Site Investigator: Grace McComsey, MD| 11100 Euclid Ave Cleveland Ohio 44106| Phone: 216-844-2739|Fax: 216-201-4607

Tool Revision History

Version Date: 12/08/16

Summary of Revision Made: Changes made to reflect NIH protocol template

Précis:

Study Title:

"Pilot Study of Zinc Supplementation and Cardiovascular risk in HIV infection"

STUDY OBJECTIVES

PRIMARY: To examine whether zinc significantly affect inflammation markers in HIV-infected subjects, we will assess changes overtime in inflammation markers, and correlate these changes with changes in zinc levels

SECONDARY:

- To examine whether zinc significantly affect microbial translocation in HIV-infected subjects, we will assess changes overtime in microbial translocation markers, and correlate these changes with changes in zinc levels

-To assess the safety and tolerability of 16 weeks of zinc in HIV-infected subjects

-To ascertain whether zinc significantly affect oxidative stress in HIV-infected subjects, we will assess changes overtime in oxidative markers, and correlate these changes with changes in zinc levels

- To examine the effects of zinc on systemic blood pressure, lipid and glucose metabolism in HIV-infected subjects with good virologic control, and correlate these changes with those of inflammatory and oxidative markers

STUDY ENDPOINTS

PRIMARY: The primary inflammation markers will be sCD14, sTNF-RI, and high sensitivity C reactive protein (hs-CRP).

SECONDARY: Microbial translocation [Primarily, Lipopolysaccharide binding protein (LBP)], zinclevel, oxidative markers, safety measures (% subjects with >Grade 2 adverse events possibly or probably to study drugs), lipid levels, insulin resistance (HOMA-IR), endoPAT, and blood pressure.

Design and Outcomes:

This is a pilot open labeled randomized double arm study, studying the efficacy and safety of Zinc therapy on cardiovascular outcomes in ≥ 18 years old HIV-infected patients , on stable ART (for at least 12 weeks) and with zinc levels ≤0.75 mg/L in the last 60 days.

Subjects will be evaluated and data will be collected according to the following schedule:

Evaluation (Weeks)	Screen	Entry*	Wk 4	Wk 10	W 16 or premature study discontinuation
Written consent form	X				
Inclusion/exclusion	X				
Family history of CVD/diabetes/HTN		X			
CVD Risk Score		X			X
Targeted physical exam/weight/blood pressure	X	X	X	X	X
Compliance assessment/safety monitoring			X	X	X
Smoking, illicit drugs and alcohol status		X			X
Dietary logs and Activity status		X			X
Height	X				
Hematology/chemistries	X	X	X	X	X
Urine β-HCG (women)	X	X	X	X	X
Fasting lipoprotein profile		X			X
Fasting insulin, glucose, HOMA-IR		X			X
Plasma inflammation markers		X			X
Microbial translocation (IFAB, LBP)		X			X
Oxidative markers		X			X
Zinc levels	X	X			X
Calcium Score		X			
DEXA		X			
Endothelial function		X			X
Resting Energy Expenditure (REE)		X			X
Stored fasting plasma, serum, urine and stool ***	X	X	X	X	X

Intervention and Duration:

The duration of the study will be 16 weeks. Patients will be given zinc gluconate capsules at a dose of 45 mg, or 90 mg, elemental zinc daily for 16 weeks. No additional follow up will occur after 16 weeks.

Sample size and population:

The total sample size is 50 patients, 25 patients will be randomized to one (45 mg) capsules and 25 patients will receive two 45 mg capsules (or 90 mg).

It has been now estimated by the World Health Organization that nearly two billion subjects may be zinc deficient in the developing countries. This is due to the fact that most of these populations consume mainly bread made of whole wheat flour, which is high in phosphate compound that decreases the absorption of both iron and zinc. The phytate to zinc molar ratio >20 in a diet is unfavorable for zinc absorption and this may lead to zinc deficiency. In the developed countries, zinc deficiency is also prevalent in the elderly population or even in younger subjects with HIV, where up to 30% of non-malnourished subjects were found to be zinc-deficient. Low concentrations of zinc are prevalent in HIV-

1–infected male and female drug users as well as other HIV-1–infected cohorts[1, 2]. Such low concentrations of plasma zinc have been linked with disease progression, independent of baseline CD4 cell count, lymphocyte concentrations and age- and calorie-adjusted dietary intake[3]. Of particular importance, low plasma zinc levels have been associated with a threefold increased risk of HIV-1–related death in HIV-1–seropositive drug users[4].

In mild to moderate deficiency of zinc, the clinical manifestations may include growth retardation in children, rough skin, poor appetite, mental lethargy, delayed wound healing, T cell-mediated immune dysfunction, and neurosensory disorders[5, 6]

Pertinent to this proposal, zinc has anti-inflammatory properties due to NFKB blockade, and is an anti-oxidant agent[7]. Zinc decrease NF-κB activation and its target genes such as TNF-α, IL-1β, and VCAM and increased the gene expression of A20 and PPAR-α, the two zinc finger proteins with anti-inflammatory properties. Thus, zinc decreased the expression of these cytokines and molecules by inhibition of NF-κB activation via A20 and PPAR-α pathways. As such, zinc supplementation has been investigated as immunomodulatory and antiinflammatory agents[5, 8, 9]

In healthy volunteers who were supplemented with 45 mg elemental zinc daily, a significant decrease in TNF-α and IL-1β messenger RNAs and TNF-α induced NF-κB DNA binding were found in isolated peripheral blood mononuclear cells in comparison to placebo treated subjects[10]. In elderly, zinc supplementation with daily 45 mg elemental zinc as gluconate for 6 months was safe, and led to significant increase in plasma antioxidant power, decrease in plasma oxidative stress marker, and decrease in the inflammation markers C-reactive protein, IL-6, MCP-1, and VCAM--1[5]. Even more, plasma zinc concentrations were inversely correlated with the changes in plasma levels of hsCRP, MCP-1, and VCAM-1 after the zinc supplementation.

The Recommended Dietary Allowance for zinc (intake of 15 mg zinc/d for men and 12 mg zinc/d women) was established in 1974 for the first time. We will use 45 mg and 90 mg elemental zinc/d as oral supplementation because it has been used safely in non-HIV studies including in elderly individuals for 1 year duration, and at this concentration, no serious adverse events were reported and copper status remained normal[5]. This is important because the only adverse effect of very high doses of oral zinc supplementation for a prolonged duration is copper deficiency.

Zinc sulfate is inexpensive, available over the counter, and has an excellent safety profile. If zinc positively influences the mechanisms postulated to play a role in HIV cardiovascular disease, this affordable treatment would become relevant to millions of people worldwide, not only for people living with HIV but also individuals suffering from other conditions of heightened inflammation such as rheumatoid arthritis, diabetes, or lupus.

Trunk fat correlates with monocyte activation and systemic inflammation: As part of a randomized placebo-controlled trial of rosuvastatin in 147 HIV-infected subjects on ART with mostly undetectable HIV-1 RNA and with LDL-cholesterol < 130 mg/dL, measurements were obtained at study entry for trunk and limb fat (by DEXA scan), monocyte activation markers (sCD14 and sCD163) and markers of systemic inflammation. Overall, 50% of subjects were on a PI-containing regimen. Trunk fat correlated with

HOMA-IR (r=0.58; p<0.0001), sCD14 (r=0.19; p=0.018), sCD163 (r=0.23; p=0.005), hs-CRP (r=0.30; p<0.0001); IL-6 (r=0.20; p=0.017), and sTNF-RI (0.26; 0.0015). The study did not measure visceral abdominal fat or whole body measurements, but nonetheless support the hypothesis that central fat accumulation in HIV is linked to monocyte activation and systemic inflammation. The effect of zinc deficiency on body composition or metabolism is unknown.

HYPOTHESIS:

In this pilot exploratory study, our hypothesis is that short term, 16 week, zinc therapy will result in improvement in mechanisms postulated to play a role in the development and progression of HIV CVD. The endpoints are

1) improved serum pro-inflammatory cytokine profile
2) reduced oxidative stress and improved serum antioxidant status
3) Increased intestinal tight junctions with decreased microbial translocation

STUDY OBJECTIVES

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SECONDARY:

- To examine whether zinc significantly affect microbial translocation in HIV-infected subjects, we will assess changes overtime in microbial translocation markers, and correlate these changes with changes in zinc levels

-To assess the safety and tolerability of 16 weeks of zinc in HIV-infected subjects

-To ascertain whether zinc significantly affect oxidative stress in HIV-infected subjects, we will assess changes overtime in oxidative markers, and correlate these changes with changes in zinc levels

- To examine the effects of zinc on systemic blood pressure, lipid and glucose metabolism in HIV-infected subjects with good virologic control, and correlate these changes with those of inflammatory and oxidative markers

STUDY ENDPOINTS

PRIMARY: The primary inflammation markers will be sCD14, sTNF-RI, and high sensitivity C reactive protein (hs-CRP).

SECONDARY: Microbial translocation [Primarily, Lipopolysaccharide binding protein (LBP)], zinclevel, oxidative markers, safety measures (% subjects with >Grade 2 adverse events poosibly or probably to study drugs), lipid levels, insulin resistance (HOMA-IR), endoPAT, and blood pressure.

INCLUSION CRITERIA:

-HIV-1 infection as documented by any licensed ELISA test kit and confirmed by Western blot at any time prior to study entry. HIV-1 culture, HIV-1 antigen, plasma HIV-1 RNA, or a second antibody test by a method other than ELISA is acceptable as an alternative confirmatory test

-Male or Female age ≥18 years

-Zinc level ≤0.75 mg/L *in the last 60 days*

-Receiving a stable antiretroviral regimen for at least the last 12 weeks prior to study entry

-Cumulative duration of antiretrovirals for at least 6 months at study entry

-Provides written informed consent and is capable of reading and comprehending the informed consent

-All women of child-bearing potential (WOCBP) must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 72 hours prior to start of study medication. WOCBP is defined as any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy), who is not postmenopausal (defined as amenorrhea ?12 consecutive months), or is on hormone replacement therapy (HRT) with documented plasma follicle-stimulating hormone level 35mLU/mL. Women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g., vasectomy), should be considered to be of child bearing potential.

-Female subjects who are not of reproductive potential (have reached menopause or undergone hysterectomy, bilateral oophorectomy or tubal ligation) or whose male partner has undergone successful vasectomy with resulting azoospermia or has azoospermia for any other reason, are eligible without requiring the use of contraception. Acceptable documentation of menopause, sterilization, and azoospermia is patient reported history.

-All subjects must not participate in a conception process (e.g. active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization), and if participating in sexual activity that could lead to pregnancy, the female subject/male partner must use condoms (male or female) in addition to one of the following forms of contraception while on study: either a spermicidal agent, diaphragm, cervical cap, IUD, or hormonal-based contraception.

-Have no plans to alter antiretroviral therapy, diet or exercise or initiate structured/strategic antiretroviral treatment interruptions.

-Documentation of an HIV-1 RNA level of ≤400 copies/mL in the last 4 months prior to study entry

-Age at least 18 years

-Able to swallow pills.

-No diarrhea or nausea/vomiting for the last month

EXCLUSION CRITERIA

All candidates meeting any of the exclusion criteria at baseline will be excluded from study participation.

Pregnancy/lactation

Presence of inflammatory condition (besides HIV) in the last month

Regular use of agents that may affect inflammation in the last 3 months. The regular use of NSAIDS, aspirin, or statins will be allowed as long as dose has been stable for the last 3 months and is not expected to change during the study.

Presence of active neoplastic diseases requiring chemotherapy and/or use of immunosuppressive drugs

Consuming supplements containing more than the DRI level of nutrients known to affect the immune response, i.e. vitamins E, C, B6, selenium, zinc, or β -carotene and unwilling to stop.

BMI <18 kg/m2.

Known cardiovascular disease

Uncontrolled diabetes

Allergy or intolerance to zinc sulfate.

Hospitalization within the previous 28 days.

Alcohol or recreational drug use which interferes with compliance

Inability or unwillingness of the individual or legal guardian/representative to give written informed consent

-Any of the following laboratory findings obtained within 14 days prior to the screening evaluation including the following:

-AST and ALT > 2.5 x ULN

-Hemoglobin < 9.0 g/dL

-GFR <50 mL/min

STUDY DESIGN

This is a pilot open labeled randomized double arm study. The duration of the study will be 16 weeks. The total sample size is 50 patients, 25 patients will be randomized to one (45 mg) capsules and 25

patients will receive two 45 mg capsules (or 90 mg). Patients will be randomized by the study pharmacist once the patient is identified and has completed screening evaluations. Patients will be given zinc gluconate capsules at a dose of 45 mg, or 90 mg, elemental zinc daily. Study drugs will be provided free of charge to study participants for the duration of their participation in the study. Antiretroviral therapy will not be provided by the study. All study visits will take place in the Special Immunology outpatient clinic. The decision for ART changes or discontinuation will be left to the primary care provider, but these changes will be documented in the study chart and will be taken into account during the analyses. Subjects will be evaluated and data will be collected according to the following schedule:

Evaluation (Weeks)	Screen	Entry*	Wk 4	Wk 10	W 16 or premature study discontinuation
Written consent form	X				
Inclusion/exclusion	X				
Family history of CVD/diabetes/HTN		X			
CVD Risk Score		X			X
Targeted physical exam/weight/blood pressure	X	X	X	X	X
Compliance assessment/safety monitoring			X	X	X
Smoking, illicit drugs and alcohol status		X			X
Dietary logs and Activity status		X			X
Height	X				
Hematology/chemistries	X	X	X	X	X
Urine β-HCG (women)	X	X	X	X	X
Fasting lipoprotein profile		X			X
Fasting insulin, glucose, HOMA-IR		X			X
Plasma inflammation markers		X			X
Microbial translocation (IFAB, LBP)		X			X
Oxidative markers		X			X
Zinc levels	X	X			X
Calcium Score		X			
DEXA		X			
Endothelial function		X			X
Resting Energy Expenditure (REE)		X			X
Stored fasting plasma, serum, urine and stool ***	X	X	X	X	X

*** Plasma, serum, urine and stool will be stored in the local laboratory in a -70 degrees freezer for potential future metabolic, cardiovascular, oxidative markers, inflammation markers, and nutrition markers. Urine will be used for possible oxidative and bone markers.

Randomization:

The randomization schedule will be performed by a statistician using SAS software to create a list based on permuted variably sized block randomization with block sizes of 4, 6 and 10 to generate 50 allocated numbers in two arms. The randomization schedule will be generated by the statisticians prior to the start of the trial by and provided to the investigational pharmacy. After being provided to the pharmacists, the randomization schedule will be maintained in a locked, secured location at the

pharmacy. Only site pharmacists can access the randomization schedule at the time of the intervention assignment.

Blinding:

Patients will be unblinded to treatment allocation (1 versus 2 zinc gluconate capsules). The research nurse, responsible for providing the patient with zinc gluconate capsules will also be unblinded to treatment assignment. They will provide the patient with the capsules (1 or 2 capsules) and assess patient's adherence to the pills. Patient's treatment as well as adherence to pills (pills count) will be collected by the research nurse and kept in a locked electronic data sheet, only they have access to. Only the percent adherence to treatment (a percentage) will be shared with the rest of research team and entered in the shared electronic database.

The research assistant as well as the principle investigator will remain blinded to treatment assignment. The research assistant will be responsible to prepare DSMP reports with the principal investigator and will include enrollment and dropout rates, protocol deviations, subjects symptoms, review of clinical, laboratory results and percent adherence to treatment, while remaining blinded to treatment allocation.

The principle investigator, as well as the DSMP board will remain blinded throughout the conduct of the clinical trial unless unblinding is warranted to optimize management of an adverse event or for other safety reasons. In these specific circumstances, the research nurse will be responsible for breaking the blind to the principal investigator. This will occur in a private location to ensure the rest of the team remains blinded.

All statistical analyses will also be done in a blinded fashion. Data will be exported to the statistician as arm A versus arm B by the research nurse to preserve blinding. For analyzing adherence, they will also only have access to the percent as opposed to pill counts the research nurse uses to calculate this.

TIMING OF EVALUATIONS

Screening

All screening evaluations to determine eligibility must be completed within 30 days prior to study entry.

Entry

Evaluations must occur within 30 days after screening evaluations. If subject shows up ill with any acute symptom, the visit will be postponed until all symptoms resolved for at least 7 days.

On-Study Evaluations

Study visits must be scheduled on the weeks indicated in the schedule of events +/- 14 days.

Premature study discontinuation

Subjects who stop study will undergo the premature study discontinuation evaluations as outlined in the Schedule of events under Week 16 visit. However if subjects stop study medication at any point during the study they will be asked to remain on study and continue to follow the procedures outlined.

DETAILED STUDY PROCEDURES BY VISITS

Screening: Before they enter, patients will be asked to visit the clinic at least once to be screened and ensure that they meet the requirements for entry into the study. Screening may take place on the same day as a normally scheduled clinic appointment, as long as subject is fasting. Before any tests can be obtained as part of this study, patients would have to decide whether or not they would like to participate in this study.

- If they choose to enroll, the informed consent form will be signed. The site investigator or a member of the study team will conduct the consenting process and a checklist indicating date and time the consent was signed, who was present, version date and if a copy was provided to participant will be filed in the subjects chart
- A targeted physical examination will be performed including height, weight and blood pressure
- Recent blood work will be reviewed to be sure that they meet the inclusion criteria. The HIV-1 RNA, and HIV testing to confirm HIV status will be obtained from the clinical chart as these are a part of routine care.
- Blood will be drawn for hematology/chemistries and zinc
- Plasma, serum, urine and stool will be collected and stored for potential future testing.
- For woman of reproductive potential, a urine sample will be taken for a pregnancy test.

If a participant does not meet inclusion criteria it will be noted in the chart with the reason for ineligibility.

If a participant meets the inclusion criteria and chooses to enroll, randomization will occur at the entry visit. The study team will notify the pharmacist which will allocate them with one of the two interventions according to the randomization schedule. Participants should start the intervention within two days of randomization allocation.

Study Entry: For entry visit, this will be fasting, as defined by no food or drink, except for plain water and medications for at least 8 hours. The following procedures will be done at entry.

- A medical history including family history
- Targeted physical exam including weight and blood pressure
- Patients will fill out a dietary, physical activity, and a substance questionnaire.
- Blood will be obtained for markers of inflammation, microbial translocation, oxidative markers, glucose and insulin, chemistries, hematology, lipids and zinc. HIV-1 RNA, and CD4 count will be obtained from the clinical chart as these are part of routine care. Some of the plasma, serum urine and stool will be stored for potential future testing such as additional tests for inflammation, cardiovascular markers, endothelial function markers, oxidative markers. These

blood samples will be batched and the tests will be done at the end of the study and are part of this study.

- For woman of reproductive potential, a urine sample will be taken for a pregnancy test.
- CVD Risk Score
- Whole body DEXA scans will be done at Entry only. Limb fat, lean body mass, trunk fat, total fat, and other measurements will be measured by dual-energy absorptiometry in the anteroposterior view (using the same Hologic scanner for all subjects). An experienced technician will scan each patient using the same machine on all study subjects.
- Resting Energy Expenditure (REE) will be done at Entry and Week 16. REE will be performed in the SIU by the dietician who is experienced with this procedure using the BodyGem and MedGem Measurement Protocol. Patients will be in a fasting state for 8 hours and nicotine free for 1 hour for this test; they will be in a rested state. The total test will take about 30 minutes in a private exam room in the SIU, while the measurement takes 5-10 minutes. A single-use mouthpiece is placed into the flow tube and the device is placed on a flat surface. Subjects will be wearing a one-time use nose clip to force only mouth breathing. The subject breathes normal while the device measures the REE.
- CT scan for Calcium scoring and pericardial fat assessment will be done at Entry only. The use of cardiac CT for CAC scoring to more accurately discriminate CVD risk has grown based upon the observation that CAC occurs almost exclusively in patients with atherosclerotic vascular disease and is not present in normal coronary arteries
- Endothelial function (EndoPAT): a non-invasive, user-independent technique using the FDA-approved EndoPAT2000 (Itamar Medical Ltd, Israel) provides a reliable and reproducible assessment of endothelial function that predicts atherosclerosis and CVD events. Using modified plethysmographic biosensors, the PAT signal is measured from the fingertip by measuring arterial pulsatile volume changes. A cuff is inflated around the upper arm to obstruct flow and released. The surge of blood flow causes endothelial dependent FMD, manifested as a reactive hyperemia. The EndoPAT® calculate the RHI, the ratio of digital pulse volume during reactive hyperemia and the baseline. In addition to the RHI, we will obtain peripheral Aix, adjusted for HR of 75, which will be compared to central Aix obtained with PWV. We have excellent track record in performing vascular studies, and carefully control for factors that could influence endoPAT; studies are performed at 24 C with the patient resting quietly. Subjects will be fasting, without smoking, caffeine, and exercise for >4 hours. Follow-up studies are completed at the same time of day as the initial study using the same equipment.

-The follow up study visits will be at 4, 10 and 16 weeks (end of study). Fasting is also mandatory for these visits. Below is the detailed events that will occur at these visits.

Week 4 and 10:

- Questionnaires about symptoms or any change in health status
- A short targeted physical exam including weight and blood pressure

- Urine pregnancy test for women
- Assess compliance/safety monitoring
- Blood will be collected for hematology/chemistries. HIV-1 RNA and CD4 count will be obtained from the clinical chart, if available.
- Plasma, serum, urine and stool will be collected and stored for potential future testing

Week 16:

- Targeted physical exam including weight and blood pressure
- Assess compliance/safety monitoring
- Questionnaire about symptoms or any change in health status, substance use and physical activity
- Dietary assessment
- Urine pregnancy test for women
- CVD Risk Score
- EndoPat
- REE
- Blood will be collected for hematology/chemistries, lipids, insulin and zinc
- Plasma, serum, urine and stool will be stored for batch testing (similar to baseline visit) to check for inflammation markers, oxidative markers, and microbial translocation. HIV-1 RNA and CD4 count will be obtained from the clinical chart.

-At visits Entry, week 4 and week 10 study medication will be dispensed by the study staff. Subjects will be asked to return all study medication at each visit and the remaining medication will be counted by the study team and returned to the investigational pharmacy. The study team will assess adherence and record in source document. Adherence will be calculated based on the number of pills dispensed and returned. Study staff will also note in the source document if patients report missed doses. Once a patient arrives at their scheduled visit, the study team will fax a prescription to the Investigational pharmacy who will receive the elemental zinc medication in bulk and dispense per patient when they arrive at their scheduled visit.

- The zinc will be purchased from Lee Silsby Compounding Pharmacy. It will be delivered directly to our investigational pharmacy at University Hospitals. The pharmacy will store the zinc according to their regular procedures, and will dispense study drugs at the time of each study follow up visits, and the number of dispensed pills will be based on the randomization arm.

TOXICITY MANAGEMENT

Only toxicities related to study drugs (zinc) will be considered. Toxicities felt to be unrelated to study drugs will be left to the discretion of the primary care provider of the study participants. AACTG grading system will be used for evaluation for Grading Adult Adverse Experiences.

-Subjects who develop a new Grade 1 adverse event or toxicity may continue study drug without alteration of the dosage. Subjects experiencing Grade 1 adverse event who choose to withdraw from the study should be encouraged to complete the study evaluations as outlined in the Schedule of events.

- For all toxicities of Grade 2 thought to be related to the study drug, the study medication can be held at the discretion of the local investigator. If unable to resume it within 3 weeks, then the subject will be taken off study drugs but will continue to be followed and undergo the evaluations as per schedule of events.

- For all toxicities Grade 3 thought to be related to study drug, study medication will be held until the toxicity grade returns to ≤ Grade 2 or to the entry value. If unable to resume study drugs for longer than 3 weeks, then the subject will be taken off study drugs but will continue to be followed and undergo the evaluations as per schedule of events.

- For any Grade 4 toxicity (confirmed X 2 values for laboratory abnormalities) regardless of the cause, subjects will be taken off study drugs but will continue to be followed and undergo the evaluations as per schedule of events. Exceptions are asymptomatic elevation of indirect bilirubin in subjects receiving indinavir or atazanavir therapy, asymptomatic elevation of CPK, or elevations in lipid levels.

CRITERIA FOR PERMANENT STUDY DISCONTINUATION

-Request by the subject to withdraw

-At the discretion of the IRB

-Pregnancy or breast-feeding

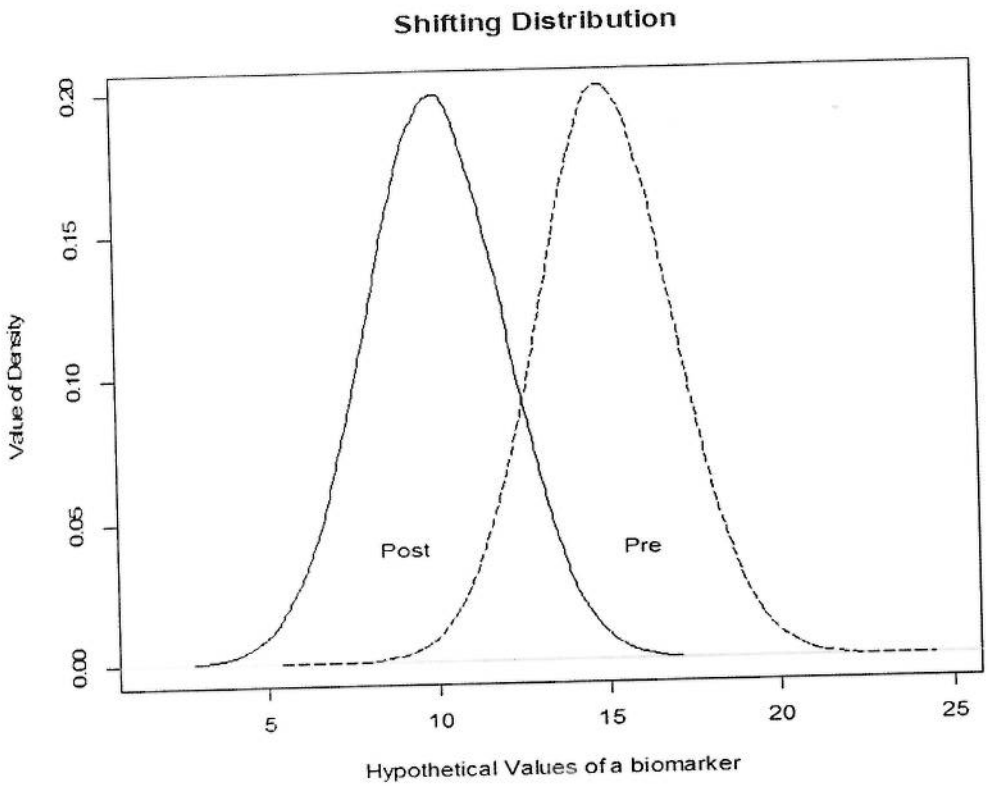
-Request of the primary care physician if s/he thinks the study is no longer in the best interest of the subject.

STATISTICAL CONSIDERATIONS

The primary Aim of this pilot study is to demonstrate that oral dosing of zinc can impact a biological signature in patients with HIV. Biological signatures (hs-CRP, sCD14, sTNF-RI, LBP) will be assessed to determine if giving the natural product (zinc gluconate at 45 mg and 90 mg) to patients with HIV results in a meaningful change in the measure.

Inter-assay variability less than 15% are generally acceptable[11]. To reduce this variability we will repeat each of these assays twice (run in duplicate) and compute average from the repeated assays performed in the same lab under same conditions conducted by the same technician. Given this set-up, the bioassays will efficiently quantify the biomarkers with less than 6% inter-assay variability which is substantially less than the general acceptable guidelines. Moreover, this inter-assay variability should be equivalent in both sets of measures obtained at baseline and after the Zinc supplementation.

We hypothesize that the distribution of at least two of the four biomarkers of primary interest (hs-CRP, sCD14, sTNF-RI and LBP) will be shifted towards left after oral supplementation compared to the baseline distribution of the biomarker, see the Figure below. This shift/change in the distribution is primarily due to the location change of a biomarker. Since the random or nuisance variations are same (although nominal) in both distributions, we will perform hypothesis tests for the systematic change in the distributions due to the treatment. We will perform Kolmogorov–Smirnov (K-S) test for the differences in distributions and median test for location change. These tests will determine significant effects of zinc supplementation on the targeted biomarkers. The K-S and median tests are global test for overall significance. We will also look at the effect of zinc supplementation on individual level.



To elicit treatment effects on each individual, we will subtract baseline value of a biomarker from the post zinc supplementation measured value of the biomarker (post minus pre). The baseline subtracted values of biomarker will have either zero (no change), positive or negative values. The baseline corrected biomarker values are in continuous nature, basically the subtracted values on the real line. For measuring significance of treatment by controlling random errors, we will perform linear regression on the baseline corrected biomarker values. This analysis has similarity with the Microarray normalization method using iterative linear regression[12]. The significant regression coefficient (intercept only) would indicate that the difference is due to the zinc supplement.

Further, for individual level impact of zinc supplementation, we will create categorical variable from the baseline subtracted biomarker values. Let's denote the number of positive, negative, and no change biomarker values by n1, n2, and n3, respectively, for a biomarker. Note that the total sample size n = n1 + n2 + n3. From this count we will compute proportions of positive, negative, and no changes. We hypothesize that most of the subtracted values will be negative. Since we want to look at the benefit of supplementation, we will compare the proportions of negative values to the proportions of positive values using McNemar's test statistic[13]. For this moderate sample size without placebo trial, we will estimate the 90% confidence interval for the proportions difference using Agresti and Min[14] methods. We will also perform logistic regression analysis on the binary change (positive vs negative) to estimate the odds ratio for the benefit of zinc supplementation. From the estimated logistic regression model will be able to predict probability of negative change (reduction in inflammation) for receiving zinc supplementation.

Effect size consideration: As above, we previously stated that we will perform significance tests for the shift of distributions of biomarkers after the zinc supplementation using Kolmogorov-Smironow (K-S) test. The amount of shift or effect size will be measured using Cohen's d value. For estimating the effect size, we used our statin study data as the preliminary data (NCT01218802). In the statin study the baseline mean (SD) of hs-CRP, log(sCD14), log(sTNF-RI), and LBP are 4.95(10.45), 7.71 (0.35), 7.46 (0.37), and 21.11(10.91), respectively. For each level of zinc supplementation (i.e. 45 mg or 90 mg zinc) we will compute the change in a biomarker by taking the difference of a biomarker measured before- and after-the zinc supplementation. For example, we will compute the difference in LBP measured at baseline and after the zinc supplementation. Similarly, differences will be computed for the other biomarkers. The sample size of 20 achieves 80% power to detect effect size (Cohen's d = mean difference/SD of differences) of 0.20 or higher using a paired t-test with a 0.10 one-sided significance level in at least two-biomarker out of the four. For the combined sample size of n=40 (both doses combined), the estimated effect size should be higher than the threshold Cohen's d = 0.20. The effect size estimation is performed using nQuary Advisor 7.0 software[15].

All analysis will be by intent to treat. All subjects, regardless of whether they remain on study drug, will continue to be followed throughout the duration of the study. It is standard practice in the HIV studies where inflammation markers are measured, that every attempt would be made to schedule study visits during a time that subjects are not acutely ill. That would mean that a visit would be rescheduled if a patient shows up sick, as acute illnesses may affect inflammation markers. A period of >7 days ill-free would be required. This happens rarely overall but if it does in the setting of this study, participants would be asked to come back after illness resolves.

HUMAN SUBJECTS

Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the University Hospitals Cleveland Medical Center CWRU IRB. A signed informed consent form will be obtained from each study subject. The informed consent form will describe the

purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, and this fact will be documented in the subject's record.

Subjects and Data Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done with coded numbers only by study staff. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB, FDA, or OHRP.

Recruitment plans

Subjects will be primarily recruited from the practice of the investigator (the Special Immunology Unit at University Hospitals Case Medical Center). All subjects seen at the Special Immunology Unit who meet the inclusion/exclusion criteria will be approached for study participation. In addition, subjects may be referred to the study by their primary care provider or their HIV provider from other HIV practices.

Study Discontinuation

The study may be discontinued at any time by the IRB as part of its duties to ensure that research subjects are protected.

Post-study Follow-up and Transition of Care

During the study period, the study subject will be encouraged to continue their routine regular follow up with their primary HIV care provider, whether in the Special Immunology Unit or in an outside HIV practice that referred them to the study. The study is not meant to replace the routine HIV care visits. After the study subject completes the study, he/she will continue to be followed by his/her primary HIV provider. There will be no additional follow-up for this study after the 16-week of the pre-defined study period. In addition, after the study subject completes the study, he/she will not be provided any further zinc supplements. If the subject primary care provider decides that it is in the best interest of the patient to be on zinc, he/she would be prescribed such supplements. The decision related to any follow-up or treatment after the study period will be left to the subject and his/her primary care provider.

BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All infectious specimens will be packaged and sent in accordance with requirements mandated by the International Air Transport Association Dangerous Goods Regulations- Packing Instruction 602.

DATA SAFETY AND MONITORING PLAN

This is a pilot study that will serve to collect preliminary data on the anti-inflammatory effects of zinc in HIV. However, an independent data and safety monitoring board will be established to include an independent HIV expert, a statistician, and a community advocate. The DSMB will meet every 6 months (or earlier as needed) to review safety data and adverse events. The DSMB will function to: review and approve plans for data and safety monitoring for this trial; to review data on a timely basis and to ensure proper conduct and progress of study; to review credentials of investigator and project staff; to make recommendations to project investigator and staff regarding issues of concern; to address adverse events. The DSMB will meet twice a year in Cleveland, Ohio or by phone conferences to review study protocols progress, and safety data. Meetings will be held in an open format, except if privileged data are discussed. At the time of the continuing review, the DSMB report will be prepared by Dr McComsey and her staff and will include enrollment and dropout rates, protocol deviations, subjects symptoms, review of clinical and laboratory results.

The DSMB will review interim data to detect evidence of adverse events to determine if the trial should continue as originally designed, should be changed or even stopped based on the data. The DSMB will evaluate the progress of the trial, including periodic assessments of the recruitment goals, protocol adherence, accrual and retention of participants. The DSMB will protect confidentiality of the study subjects.

The Principal Investigator will report all > Grade 2 adverse effects deemed to be possibly, probably or definitely related to study participation to the University Hospitals Cleveland Medical Center CWRU IRB within one week in writing. All >1 Grade 1 adverse effects deemed to be possibly, probably or definitely related to study participation will be collected. The Principal Investigator, investigators, and study nurse will prepare every year an update to renew the University Hospitals Cleveland Medical Center CWRU IRB approval of the clinical studies. In this renewal, investigators will inform the IRB about adverse effects Grade > 1 noted during the performance of the study. Accrual, retention, and data quality and timeliness will be monitored by the Principal Investigator.

Protocol Deviations: All deviations will be documented in the source document and the IRB will review all protocol deviations. Any deviation that may affect study conduct or safety will promptly be reported within 72 hours.

Quality Control Committee: Not applicable

Adverse Events

Definitions

The definitions of Adverse Events (AEs) and Serious Adverse Events (SAEs) are given below. It is of the utmost importance that all staff involved in the study be familiar with the content of this section. The principal investigator is responsible for ensuring this.

Adverse Event

An Adverse Event (AE) is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, ECG). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

Any detrimental change in a patient's condition subsequent to them entering the study and during the follow-up period should be considered an AE. When there is a deterioration in the condition for which the study treatment is being used, there may be uncertainty as to whether this is lack of efficacy or an AE. In such cases, unless the reporting physician considers that study treatment contributed to the deterioration or local regulations state to the contrary, the deterioration should be considered a lack of efficacy. Signs and symptoms of disease progression are therefore not considered AEs.

Serious Adverse Event

- A Serious Adverse Event (SAE) is an AE occurring during any study phase (eg, run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above.

Any event or hospitalization that is unequivocally due to progression of disease, as determined by the investigator, must not be reported as an SAE.

The causality of SAEs (their relationship to all study treatment) will be assessed by the investigator(s)

Reporting of Adverse Events

When recording/reporting AEs, the use of diagnoses is preferred (when possible) to recording/reporting a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded/reported separately.

Illiterate Subjects

Subjects with all levels of literacy will be eligible for this study. The consent document will be read to those volunteers with less than an 8th grade equivalent level of literacy. Subsequently, the informed consent will be signed by the volunteer making their mark in the signature section in order to document their understanding. A witness will be present to confirm the consent process has taken place. Both the witness and person conducting the consent process will sign and date the consent. The investigator obtaining consent will ask each subject to reiterate what will be required from them, risks and benefits, and their rights as a participant in order to ensure their full understanding of the study.

Non-English Speaking Subjects

Subjects who do not understand or speak English will also be eligible for this study. The consent form will be read to those non-english speaking study candidates in their primary language by a translator from the University Hospitals of Cleveland translation/International Visitors Center. A witness (who speaks English and the study subject's language) will be present to confirm the consent process has taken place. Both the witness and person conducting the consent process will sign and date the consent. The investigator obtaining consent will ask the study candidate via the translator to reiterate what will be required from him/her, risks and benefits, and his/her rights as a participant in order to ensure their full understanding of the study.

BENEFITS

There may be no direct benefit to patients from participating in this study. Their participation in the study will allow collection of valuable information about the potential benefits of zinc in persons infected with the HIV virus. This information may be useful to them and other people with HIV disease.

ALTERNATIVES

Alternatives to participation in this study are not to participate and to receive the standard of care from the patient's primary care doctor.

COST TO PARTICIPANTS

There is no cost to participants for the study related clinic visits, examinations or laboratory test required by this study. Medical costs of other treatment or examinations outside of the study will be the responsibility of the patient or their insurance company. Patients will be provided zinc supplements free of charge for the entire duration of the study. The study drug will not be provided after they finish the study (whether they complete the study or prematurely stop study participation).

PAYMENT TO SUBJECTS

For completed study visits screening, week 4 and week 10, patients will receive $25.00 that day. Entry and week 16, patients will receive $50.00 that day (total of $175 for completing the entire study). This payment will help cover the expense of childcare, transportation and time off work that patients may incur as a result of being in this study. To help with the cost of gas, for participants traveling > 20-40 miles one way for their appointments, they will also be given a $15.00 gas card to cover the cost of the

transportation. Participants traveling > 40 miles one way for their appointments will also be given a $30.00 gas card to cover the cost of transportation. . Subjects will be asked to fast (nothing to eat or drink for 8 hours prior to visit) at entry, week 4, week 10 and week 16, therefore a $5.00 meal voucher will be provided for subjects to use in the University Hospitals cafeteria. In addition either one all day RTA pass will be provided or a parking voucher to use in designated University Hospitals parking garages will be provided to cover the cost of transportation.

RISKS AND DISCOMFORTS: The risks for taking part in this study are:

Blood Draw: Risks associated with drawing blood include: pain, bleeding, and bruising at the site of the blood draw. Other rare risks include: lightheadedness and/or fainting or infection at the site.

EndoPat: This is a painless imaging test that has no short or long-term risks. The endoPAT test may be mildly to moderately uncomfortable because of the blood pressure cuff that is applied to the patients' arm tightly.

Fasting: Some individuals find fasting to be bothersome. It may make some individuals feel anxious, irritable, or hungry. Patients who are required to take their morning medications with food should wait until after the visit has been completed to take their medications.

Pregnancy : Zinc may not be safe for unborn babies. If patients are having sex that could lead to pregnancy, they must agree not to become pregnant or make a woman pregnant. Because of the risk involved, patients and their partner must use two methods of birth control that they discuss with the study staff. They must continue to use both methods until 6 weeks after stopping study drug. They may choose two of the birth control methods listed below:

- Birth control drugs that prevent pregnancy given by pills, shots, intra-vaginal ring or placed on or under the skin
- Male or female condoms with or without a cream or gel that kills sperm
- Diaphragm or cervical cap with a cream or gel that kills sperm
- Intrauterine device (IUD)

If women can become pregnant, they must have a pregnancy test before they enter this study. The pregnancy test must be negative. In addition, is study subjects think they may be pregnant at any time during the study, they are to tell the study staff right away. In the event that a patient becomes pregnant while on study, they will be taken off study, and no further evaluations or tests will be performed as part of the study.

Zinc Supplementation: At the doses used, uncommon side effects include nausea, vomiting, and abdominal pain (gastritis). At higher doses and when used for a long periods (years), copper deficiency

and related anemia may happen. This is extremely unlikely since we are only giving 45 mg or 90 mg daily for up to 16 weeks. Such doses have been shown to be safe even when given for 12 months to elderly frail subjects. In addition, in diseases such as Wilson's disease, zinc is given at a dose of 50 mg three times daily for prolonged durations. We will closely monitor subjects who will receive blood draws for chemistries, hematology and liver enzymes.

OTHER RISKS: Patients are informed to tell their study doctor or study nurse about all other drugs they are currently taking including non-prescription medications, alcohol, recreational, and herbal products. These drugs, if taken with the study medication, can result in dangerous interactions. If patients have questions about the drug that will be used in this study and the potential for interaction with other drugs that they take, they are instructed to ask their study doctor to provide additional information.

In addition to the risks and discomforts listed here, there may be others that are currently not known.

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