# Impact of parasitic infections on intestinal epithelial barrier and immune activation among persons living with HIV in Lilongwe, Malawi

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**Title:** Impact of parasitic infections on intestinal epithelial barrier and immune activation among persons living with HIV in Lilongwe, Malawi

**Abbreviated study name: HAPI** (**H**IV **A**nd **P**arasitic **I**nfection) study

**Background:**
In sub-Saharan Africa (SSA), more than 22 million people are estimated to be co-infected with HIV and helminths[1] and studies within SSA have reported 14-25% of persons living with HIV (PLWH) coinfected with pathogenic intestinal protozoa[2,3]. A prior study from Lilongwe, Malawi published in 2007 reported a prevalence of 30% of PLWH coinfected with intestinal parasitic infection when including both helminths and pathogenic intestinal protozoa[4]. Although treatable, intestinal parasitic infections are underdiagnosed due to lack of testing and inadequate sensitivity of currently used techniques such as light microscopy. Furthermore, reinfection in endemic regions is common. Consequently, co-infection remains highly prevalent.

$CD4^+$ T cell recovery has been shown to be lower in patients from sub-Saharan Africa compared to Europeans early after starting cART after adjusting for age, sex, initial regimen, pre-ART $CD4^+$ T-cell count, and HIV RNA[5-7]. This is an indication that factors other than initial HIV disease state contribute to HIV disease progression in these resource-limited, parasitic-endemic settings. A study in Uganda- using the same stool PCR test that we will use in our proposed study- demonstrated significantly lower levels of CD4 cells in patients co-infected with hookworm, suggesting that intestinal parasitic infection could be contributing to the low CD4 count and delayed recovery in resource-limited settings[8].

Soluble CD14 is a marker of microbial translocation, released upon LPS stimulation[9]. Both sCD14 and soluble CD163 (sCD163) are markers of macrophage and monocyte activation[9-11]. Intestinal fatty acid binding protein (I-FABP) is a marker of intestinal permeability and enterocyte turnover[10]. Increased levels of each of these- soluble CD14 (sCD14), soluble CD163 (sCD163), and (I-FABP)- have been shown to predict morbidity and mortality in treated HIV infection, including cardiac disease and neurocognitive decline[9-14.] Preliminary data from a study in Houston, Texas, showed increased levels of sCD14 in PLWH co-infected with *Strongyloides stercoralis* when compared to *Strongyloides* negative PLWH, and a correlation of *Strongyloides* IgG levels with sCD14 and sCD163 levels[15].

Characterizing the effects of intestinal parasitic infections on HIV disease will provide clarification as to whether periodic deworming would improve CD4+ T cell recovery and decrease inflammation in PLWH, and will highlight key pathways to explore for optimizing the health of PLWH worldwide. The impact on systemic inflammation and intestinal damage may have consequences for the long-term risk of serious non-AIDS events.

**Objectives:**
The **overall goal** of our research program is to determine if periodic deworming of persons living with HIV in intestinal parasite-endemic regions will lead to decreased morbidity and mortality associated with HIV by reducing immune activation and intestinal damage associated with these diseases. Our hypothesis for this project is that intestinal parasitic infections contribute to a modifiable pro-inflammatory state in PLWH.

**Aim 1:** Determine the prevalence of intestinal parasitic infections in PLWH receiving care at an HIV-treatment center in Lilongwe, Malawi using a highly sensitive multi-parallel stool PCR test. Hypothesis: highly sensitive stool PCR testing will demonstrate that disease burden of parasitic infection in PLWH in Malawi is higher than historically reported based on light microscopy.

**Aim 2:** Determine the impact of parasitic infection and eradication on intestinal damage and immune activation by measuring sCD14, sCD163, and intestinal fatty acid binding protein (I-FABP) in PLWH before and after treatment of parasitic co-infection. Hypothesis: plasma biomarkers reflecting intestinal damage and immune activation are elevated in those with HIV and parasitic co-infection compared with parasite negative participants with HIV, and these biomarkers decrease with anti-parasitic treatment.

Few studies have evaluated the effect of eradication of intestinal parasitic infection on PLWH on suppressive ART[15-16], and fewer have been prospective studies. We will use a modern molecular diagnostic and quantitative technique, the multi-parallel real-time quantitative PCR (qPCR), which detects and quantifies the burden of 9 common GI parasites including helminths and protozoa simultaneously, with more than 10 times higher sensitivity compared to traditional microscopy[8]. This assay has been optimized at the US National Institutes of Health (NIH) and field tested in Ecuador, Colombia, Mozambique, Argentina, and Uganda[8,17,18]. Our test will be modified to include PCR for *Schistosoma mansoni,* given the high prevalence of intestinal schistosomiasis in Malawi. This improved sensitivity will overcome the deficiencies of microscopy that have plagued previous studies, resulting in under-diagnosis of parasitic infections and contamination of parasite-negative groups with undiagnosed parasite-infected persons. This approach will facilitate learning the prevalence of parasitic infections, maximize our ability to ensure a truly parasite-uninfected control group, and enable us to assess the impact not only of the presence of intestinal parasites but also the impact of intestinal parasite burden on HIV infection. Comparison of sCD14, sCD163, and I-FABP levels in PLWH with and without parasitic infections, and repeated measurements after eradication will provide data on the impact of intestinal parasitic infections on systemic immune activation and intestinal permeability. Outside of preliminary data from our study performed in Houston, Texas[15], these markers of systemic immune activation and intestinal damage have not been explored in PLWH on ART with parasitic coinfection.

In accordance with the SMART objectives, our goals are specific, measurable, achievable, relevant, and time-bound. The laboratory data obtained by qPCR, *Strongyloides stercoralis* IgG, sCD14, sCD163, and I-FABP are specific and easily measured. The estimated patient enrollment is achievable in the outlined period of time based on the number of patients that regularly receive clinical care at the participating clinics. Once the data are obtained, our research question is achievable with the power calculations outlined above. The relevance of our research question is highlighted by the fact that intestinal parasitic infections may be a modifiable pro-inflammatory state in PLWH contributing to increased morbidity and mortality of this vulnerable population.

**Literature Review:**

**I. HIV and intestinal parasitic coinfection**

In sub-Saharan Africa, more than 22 million people are estimated to be co-infected with HIV and helminths[1] and regions of SSA have reported 14-25% of PLWH coinfected with pathogenic intestinal protozoa[2,3]. One study from Ethiopia reported as many as 59.8% of PLWH coinfected with intestinal parasitic infections when including both helminths and protozoa[19]. A prior study from Lilongwe, Malawi published in 2007 reported a prevalence of 30% of PLWH coinfected with intestinal parasitic infection[4].

Although treatable, intestinal parasitic infections are underdiagnosed due to lack of testing and inadequate sensitivity of currently used techniques such as light microscopy. Furthermore, reinfection in endemic regions is common. Consequently, co-infection remains highly prevalent.

**II. Delayed CD+ T cell recovery in PLWH in intestinal parasite-endemic regions**

$CD4^+$ T cell recovery has been shown to be lower in patients from sub-Saharan Africa compared to Europeans early after starting cART[5-6]. $CD4^+$ T-cell counts after 3 years of ART were higher in people from North America (529 cells/$mm^3$) compared to East Africa (437 cells/$mm^3$), West Africa (494 cells/$mm^3$), Southern Africa (515 cells/$mm^3$), and Asia (503 cells/$mm^3$) after adjusting for age, sex, initial regimen, pre-ART $CD4^+$ T-cell count, and HIV RNA[20]. In a study from East Africa, 63% of adults had $CD4^+$ T-cell counts < 500 cells/$mm^3$ and 40% had CD4+ T-cells <350cells/$mm^3$ after 5 years of ART[5]. Similarly, in a Ugandan cohort, 49.1% of adults had $CD4^+$ T-cells ≤ 400 cells/$mm^3$ after 5 years of ART[6]. Five years of ART increased $CD4^+$ T-cell counts from a median of 114 cells/$mm^3$ to 395 cells/$mm^3$ in a consortium of participants from low-income countries in Africa, Latin America, and Asia[21]. This is an indication that factors other than initial HIV disease state contribute to HIV disease progression in (PLWH) in these resource-limited, parasitic-endemic settings.

**III. Impact of parasitic infection on the immune system**

Parasitic infections induce Th2 immune responses, characterized by high IgE titers and eosinophil recruitment[22,23]. Consequently, the Th1 response in someone with parasitic infection is often impaired[24-26]. One study showed that mice with chronic infection with the roundworm *Trichinella spiralis* and acute norovirus infection, the frequency and polyfunctionality of norovirus-specific $CD4^+$ and $CD8^+$ T-cells were decreased and virus burden increased compared to non-*Trichinella* infected mice[27]. Mice with chronic infection with the hookworm *Heligmosomoides polygyrus bakeri* had impaired antigen-specific and polyfunctional responses to norovirus[27]. Similar results were observed with influenza virus in *Trichinella spiralis* infection[26]. These data indicate that parasitic infections impair virus-specific $CD4^+$ and $CD8^+$ T-cell responses and may facilitate virus replication.

Intestinal parasitic infections induce immune activation as demonstrated by the following studies: *Trichuris* infection upregulates CCR5 expression on $CD4^+$ T-cells[28], and hookworm, *Trichuris,* and *Ascaris* induce HLA-DR expression on $CD4^+$ and $CD8^+$ T-cells[28-30]*. Trichuris* and *Ascaris* treatment downregulates CCR5 expression on $CD4^+$ T-cells and HLA-DR expression on $CD8^+$ T-cells[17]. Hookworm also decreases $CD3^+$ and $CD4^+$ populations likely due to increased T-cell apoptosis and decreased T-cell proliferation[29-32]. Among HIV-uninfected South Africans, the frequency of HLA-$DR^+$ $CD8^+$ T-cells and expression of CCR5 on $CD4^+$ T-cells decreased 3 months after albendazole and praziquantel treatment of hookworm and *Trichuris* infection[28]. In summary, intestinal parasitic infections seem to increase T-cell activation, increase CCR5 expression, induce T-cell apoptosis, and suppress T-cell proliferation.

## IV. Potential impact of parasitic infections on HIV

Recurrent enteric parasitic infections cause impaired gut healing[29,30] and increased microbial translocation[30-32], increased activation of macrophages[33], and intestinal fibrosis[34,35]. Parasitic infections increase $CCR5^+$ $CD4^+$ T-cell frequency, creating more HIV targets in HIV-infected persons[16,26]. Parasitic infections also increase $CD8^+$ T-cell activation[24]. Malnutrition, weight loss, chronic diarrhea, and changed immune responses caused by the intestinal parasitic infections may further accelerate HIV/AIDS progression[1,36-39]. In a reciprocal manner, HIV infection may impair the immune system's ability to defend against parasitic infections[40,41].

Some studies have shown that patients living with HIV (PLWH) with parasitic coinfection have weakened CD4 recovery[8,30,42,43] and/or higher HIV viral loads[44,45] compared to those without parasitic coinfection, yet the data are inconsistent. HIV-infected persons in Southern Ethiopia not taking ART who were treated for *Ascaris lumbricoides* had a significant increase in $CD4^+$ T-cells from 469 to 551 cells/$mm^3$ at 6 months, compared to a non-significant decrease in helminth-uninfected participants[46]. Similar results were observed in a randomized, double-blind, placebo-controlled trial in ART-naive Kenyans treated for *Ascaris lumbricoides*[47]. A study in Uganda- using the same stool PCR test that we will use in our proposed study- demonstrated significantly lower levels of CD4 cells in patients co-infected with hookworm, suggesting that intestinal parasitic infections could be contributing to the low CD4 count and delayed recovery in resource-limited settings[8]. Higher HIV viral loads were observed in pregnant women with hookworm or *Trichuris* infections[44]. Deworming of *T. trichiura* or hookworm in an observational study increased $CD4^+$ T-cell counts and decreased the percentage of pregnant women on ART with detectable HIV viral load[45].

In contrast to the studies above, meta-analysis of randomized controlled trials of antiparasitic medications or placebo in HIV-infected people with unknown parasitic infection status showed no significant effect of one-time or repeated deworming on viral load or $CD4^+$ T-cell count in people not taking suppressive ART[48]. A retrospective, observational study, empiric deworming did not increase $CD4^+$ T-cell counts among Ugandans on ART except when restricted to women in the first year of ART[49]. A study published in 2007 conducted in outpatient clinics in the same setting as our study- Lilongwe, Malawi- found no improvement of HIV viral load after treatment of parasitic infection in PLWH[4].

Even with the studies above, there are gaps that remain in the question of the impact of intestinal parasitic infection on HIV. Few of the studies are prospective, controlled trials involving treatment of intestinal parasitic infection. Additionally, most of the studies above used low-sensitivity light microscopy to identify those with parasitic infection. Given the low sensitivity of light microscopy, the cohort of patients identified as parasite-

negative likely included a notable number of patients with unidentified parasitic infections. Third, studies of parasitic infections in PLWH *on suppressive ART* are limited. Our proposed study will help to address the gaps identified in this topic of PLWH and parasitic coinfection.

**V. Impact of chronic immune activation and intestinal damage on morbidity of PLWH**

In HIV infection, enterocyte loss, tight junction destruction, $CD4^+$ T-cell depletion, decreased intestinal mucosal IgA, bacterial overgrowth, and dysbiosis result in increased translocation of pro-inflammatory bacterial products such as LPS[9,50]. Non-human primate models have illustrated that this gut destruction and microbial translocation begins in the first week of infection[51,52]. Increased microbial translocation results in increased T-cell and monocyte activation, increased production and recruitment of HIV target $CD4^+$ T-cells, and increased lymphatic tissue fibrosis[50,52,53]. Early suppression of microbial translocation and inflammation in SIV infection reduces immune activation, decreases SIV replication, and preserves intestinal $CD4^+$ T cells[54]. In sum, increased microbial translocation is a key driver of immune activation and inflammation in HIV infection.

Soluble CD14 (sCD14) is a marker of microbial translocation, released upon LPS stimulation[9]. Both sCD14 and soluble CD163 (sCD163) are markers of macrophage and monocyte activation[9-11]. Intestinal fatty acid binding protein (I-FABP) is a marker of intestinal permeability and enterocyte turnover[10]. Increased levels of each of these- soluble CD14 (sCD14), soluble CD163 (sCD163), and (I-FABP)- have been shown to predict morbidity and mortality in treated HIV infection, including cardiac disease and neurocognitive decline[9-14]. One study analyzing 74 participants from the Strategies for Management of Antiretroviral Treatment (SMART) trial who died and 148 matched controls found that participants with the highest quartile of soluble CD14 (sCD14) levels had a 6-fold increased risk of death compared to participants with the lowest quartile, even after adjusting for $CD4^+$ T-cell count and other inflammatory markers[9].

HIV-uninfected South Indians with hookworm have higher LPS, sCD14, endotoxin core IgG, and I-FABP levels compared to hookworm-negative persons from the same village, and LPS and sCD14 levels decreased after documented parasite clearance with treatment[55]. Preliminary data from our pilot study in Houston, Texas, showed increased levels of sCD14 in PLWH co-infected with *Strongyloides stercoralis* when compared to *Strongyloides* negative PLWH, and a correlation of *Strongyloides* IgG levels with sCD14 and sCD163 levels[15].

As demonstrated in the literature referenced above, HIV infection and recurrent enteric parasitic infections have each been shown to be independent causes of intestinal damage, increased microbial translocation from the gut, and immune activation; all of which contribute to a pro-inflammatory state that may drive chronic diseases more prevalent in the HIV-positive population. Characterizing the effects of intestinal parasitic infections on HIV disease will provide clarification as to whether periodic deworming would improve CD4+ T cell recovery and decrease inflammation in PLWH, and will highlight key pathways to explore in order to optimize the health of PLWH worldwide. The impact of parasitic infection on systemic inflammation and intestinal damage may have consequences for the long-term risk of serious non-AIDS events.

## Methodology

### Research Design

This is a prospective study in which participants will be enrolled in outpatient HIV clinics associated with Kamuzu Central Hospital in Lilongwe, Malawi, where there are over 25,000 patients in care with over 90% virally suppressed on ART.

### Subjects and Methods

Inclusion criteria include the following: Age ≥ 18 years; HIV-1 infection; currently living in Malawi; on ART ≥ 1 year with undetectable HIV RNA level at the last evaluation, and willingness to be treated with anti-parasitic therapy if infection with intestinal parasite is identified. Exclusion criteria include the following: Inflammatory bowel disease; gastrointestinal tract malignancy; major intestinal surgery during prior 2 years; use of antibiotics other than prophylaxis with trimethoprim-sulfamethoxazole within 60 days of screening; use of antiparasitic medication (ex- albendazole, praziquantel) in the last year; coinfection with

*Mycobacterium tuberculosis*; pregnancy, breastfeeding mother, or planning pregnancy. The last two are included in exclusion criteria because of the potential risk of teratogenicity with the antiparasitic medications used for treatment and because these can affect the markers of immune activation that we are measuring as a part of the study.

Any subject meeting inclusion criteria and lacking exclusion criteria who is currently receiving care at the clinics affiliated with Kamuzu Central Hospital or Bwaila Hospital will be eligible to participate in this study. Subjects will be given time to ask questions, and if agreeable they will sign consent and then study procedures will be initiated. The investigator will keep the original consent form as part of the study records, and a copy will be provided to the subjects. A pregnancy test will be performed on all women of childbearing years.

After informed consent is signed, a total of 10ml of blood, 20g stool sample, and 20mL urine sample will be collected. Subjects will be given an explanation about hygienic collection of stool samples. They will be given a clean, plastic, wide-mouthed, leak-proof container (of about 125 mL) with a snap-on lid. In addition, each subject will receive a pair of gloves and a small biohazard plastic bag. All subjects will be asked to put 5-25 g of stool in the container (which is approximately 1/5 of the volume content), close the lid and return the container to investigators in the biohazard bag. Each participant will be asked a series of questions (Participant information sheet attached).

Clinical variables including age, sex, CD4+ T-cell count, and CD4% will be collected from the participant's medical chart.

Blood samples will be collected in EDTA-blood collection tubes and centrifuged, then serum will be frozen at -80 degrees at UNC Project Malawi until transport to Houston, Texas. Stool samples will be frozen at -80 degrees until transport to Houston, Texas. Coded serum samples- without personal identifiers- will be sent to the laboratory of Dr. Rojelio Mejia at the National School of Tropical Medicine Baylor College of Medicine for determination of levels of immune activation and gut mucosal impairment (sCD14, sCD163, and I-FABP) and *Strongyloides stercoralis* IgG. Stool samples will be processed by stool microscopy in the local UNC Project Malawi laboratory, then the remaining sample will be transported to Houston, Texas to be processed by qPCR for detection of 10 different parasites and quantification of parasite burden at the Laboratory of Parasitology National School of Tropical Medicine Baylor College of Medicine. Urine samples will be brought to UNC Project Malawi for processing and evaluation by microscopy to look for *Schistosoma haematobium*.

The COVID-19 precautions followed by the study team are as follows: all subjects will complete a clinical questionnaire before entry into the clinic. Only those with low risk for current infection with SARS-CoV-2 will be approached for the study. All clinical team members will be wearing gloves and a surgical mask at the time of participant enrollment. All patients are required to wear a mask in the clinic and will be given gloves when they are given the stool sample collection kit.

After enrollment is completed, the serum and stool samples will be shipped to Houston, Texas. Stool samples will be processed by multi-parallel real-time quantitative PCR (qPCR) for detection of 9 different parasites and quantification of parasite burden. Parasites to be evaluated include *Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Schistosoma mansonii, Strongyloides stercoralis, Taenia solium, Trichuris trichiura, Entamoeba histolytica, and Giardia lamblia.* Serum will be processed for *Strongyloides stercoralis* IgG, sCD14, sCD163, and I-FABP.

Participants that test positive for parasitic infection will be contacted and appropriate treatment administered according to the local standard of care. Albendazole single 400mg dose will be given for infection with *Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Trichuris trichiura*. Albendazole 400mg daily for 5 days will be given for *Strongyloides stercoralis*. Praziquantel single dose 40mg/kg will be given for infection with *Schistosoma mansoni* and *Schistosoma haematobium*. Praziquantel single dose 10mg/kg will be given to treat intestinal infection with *Taenia solium*.

Metronidazole 500mg two times a day x5 days for *Giardia lamblia* and 500mg three times a day x7 days for *Entamoeba histolytica*.

Female participants that test positive for parasitic infection will have a repeat pregnancy test before administration of antiparasitic medication. If newly pregnant on repeat testing, the study team will be referred to her regular medical provider for evaluation, review of diagnostic tests performed by the study, and for potential treatment with antiparasitic medication. Any participant found to be pregnant at this stage will not be included in the comparison of markers of immune activation or the follow up visit since pregnancy can affect these markers of chronic immune activation. Follow up appointments for all non-pregnant participants will be performed 8-12 weeks after treatment and will include repeated blood and stool sample collection. Pregnancy test will be repeated at the follow-up visit. Any participant found to be pregnant at this stage will not be included in the comparison of markers of immune activation since pregnancy can affect these markers of chronic immune activation. The decision for post-treatment period of 8-12 weeks was made based on prior studies of sCD14[56] and other inflammatory markers[57] that used a time frame of 12 weeks between intervention and repeat measurement. A third study showed a decrease in sCD163 in the first eight weeks of treatment for HCV, but no change in either sCD14 or sCD163 from week 8 to week 24 following treatment for HCV[58].

We anticipate enrollment of 100 patients receiving care at the participating clinics during the recruitment period. With an estimated intestinal parasite prevalence of 30%, we predict 30 cases and 70 controls will be enrolled. This estimate is based on a study with similar patient population in the same region in 2007 which reported 17.3% of participants infected with helminth infections and 12.8% with pathogenic protozoal infections[4]. Our prevalence may be higher than this prior study given the increased sensitivity of our stool PCR test. Participants found to be positive at both the initial and follow up visit will be considered reinfected rather than treatment failure. These will be included in the analysis of prevalence, but the change in markers of immune activation will not be measured in this group since parasite clearance not established. Using Student's unpaired t-test to compare mean values of biomarkers between study groups, we will have 80% power to detect a difference of 0.434 $x10^6$ pg/ml, 0.56 mg/l, and 598 pg/ml between groups for biomarkers sCD14, sCD163, and I-FABP, respectively with effect sizes within the range of prior studies[9,14,59]. Using paired t-tests to compare pre- and post-treatment biomarker levels, we will have 80% power to detect post-treatment changes of 0.317 $x10^6$/ml, 0.41 mg/l, and 435 pg/ml in sCD14, CD163, and I-FABP respectively[60]. A sample size less than 100 increases the chance of a type II error where a true difference in markers of chronic inflammation does not reach clinical significance. A sample size of greater than 100 should not be necessary given the estimated findings in our patient population and the data presented by the studies cited above. A sample size of 100 should be easily attainable in a reasonable amount of time (4-8 weeks) without the unnecessary use of additional resources.

Clinical variables including age, sex, and most recent CD4 count will be recorded. Clinical predictors of parasitic infection (eg CD4%) will be determined using multivariable logistical regression. Univariable linear regression will be used to determine associations between markers of immune activation (continuous outcome variable) and predictors including the clinical variables above as well as presence of multiple parasitic infections.

**Study visits:**

- v0= Screening visit: includes explanation of study, inclusion/exclusion criteria, sign ICF
- v1= Baseline visit: complete CRF; collection of blood, stool, and urine samples
- v2= Treatment visit: only for those participants positive for parasitic infection
- v3= Post-treatment/follow-up visit performed 8-12 weeks after administration of antiparasitic therapy to those participants found to be parasite-positive. Sample collection from controls (parasite-negative participants) will be done in a similar time window.

*screening and baseline visits can be done the same day
**Participants positive for parasitic infection will complete v0, v1, v2, v3. Participants negative for

parasitic infection will not have v2. Full participation in the study for a parasite negative participant includes completion of v0, v1, v3.
***Participants will be reimbursed for v1, v2, v3.

**Ethical Considerations**

In addition to the Malawi National Health Sciences Research Committee, we will submit for IRB approval from University of North Carolina School of Medicine, and the National School of Tropical Medicine at Baylor College of Medicine. Participation in the study will be voluntary. All study participants will be explained the study design and all questions answered before the participant provides written, informed consent.

Neither the investigator, nor any research team member will coerce or unduly influence nor specifically target vulnerable subjects to participate in the study. Research subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time. Subjects will be given time to ask questions, and if agreeable they will sign consent and then study procedures will be initiated. The investigator will keep the original consent form as part of the study records, and a copy will be provided to the subjects. A Chichewa translator will be present for the entirety of each interaction with each participant.

The possibility of a breach in confidentiality will be minimized by limiting the number of people that will have access to the information, the use of coded data, and the stringent security measures that will be utilized. Only Dr. Reimer-McAtee and members of the research team will have access to the patient clinical data. The Participant Information Sheet with clinical information will only have the participant codes and deidentified clinical information. The informed consent forms and the “link-log form” with patient names and patient codes will be kept in a locked cabinet at UNC Project Malawi. Information entered into the electronic database will only include the participant identification number. The patient names will not be included in the electronic database. The database will not be saved on personal computers, rather will only be saved to a secure UNC server and will be password protected.

**Work Plan**

We plan to begin enrollment of participants starting January 2022. We estimate that 2-3 months will be sufficient to recruit 100 participants. After 100 participants are enrolled and samples collected, we will send samples to Houston, Texas. We expect it will take 1-2 months to process all of the samples in the laboratory of Dr. Mejia. Follow up visits will take place 2-3 months after participants with parasitic infections were treated. Once follow up visits completed and samples obtained, the second shipment of samples will be sent to Houston, Texas. We expect another 1-2 months for the processing of samples in the laboratory of Dr. Mejia. Once all samples are processed, laboratory analysis can be performed. A manuscript will be prepared and submitted for publication. An infectious diseases journal will be the target for publication of the manuscript. The NHSRC will be provided a copy of the manuscript. Additionally, we plan to present our findings at both a local and an international academic conference.

## VI. References


1. Sangare LR, Herrin BR, John-Stewart G, Walson JL. Species-specific treatment effects of helminth/HIV-1 co-infection: a systematic review and meta-analysis. Parasitology. 2011;138(12):1546-58. Epub 2011/07/07. doi: 10.1017/s0031182011000357. PubMed PMID: 21729353;
2. Wang ZD, Liu Q, Liu HH, Li S, Zhang L, Zhao YK, Zhu XQ. Prevalence of Cryptosporidium, microsporidia and Isospora infection in HIV-infected people: a global systematic review and meta-analysis. Parasit Vectors. 2018 Jan 9;11(1):28. doi: 10.1186/s13071-017-2558-x. PMID: 29316950; PMCID: PMC5759777.
3. Shimelis T, Tassachew Y, Lambiyo T. Cryptosporidium and other intestinal parasitic infections among HIV patients in southern Ethiopia: significance of improved HIV-related care. Parasit Vectors. 2016 May 10;9(1):270. doi: 10.1186/s13071-016-1554-x. PMID: 27165271; PMCID: PMC4862162.
4. Hosseinipour MC, Napravnik S, Joaki G, Gama S, Mbeye N, Banda B, Martinson F, Hoffman I, Cohen MS. HIV and parasitic infection and the effect of treatment among adult outpatients in Malawi. J Infect Dis. 2007 May 1;195(9):1278-82. doi: 10.1086/513274. Epub 2007 Mar 20. PMID: 17396996.
5. Nakanjako D, Kiragga AN, Musick B, Yiannoutsos C, Wools-Kaloustian K, Diero L, Oyaro P, Lugina E, Ssali JC, Kambugu A, Easterbrook P. Frequency and impact of suboptimal immune recovery on first-line antiretroviral therapy (ART) within the IeDEA- East Africa cohort. AIDS (London, England). 2016. Epub 2016/03/10. doi: 10.1097/qad.0000000000001085. PubMed PMID: 26959510.
6. Castelnuovo B, Kiragga A, Musaazi J, Sempa J, Mubiru F, Wanyama J, Wandera B, Kamya MR, Kambugu A. Outcomes in a Cohort of Patients Started on Antiretroviral Treatment and Followed up for a Decade in an Urban Clinic in Uganda. PloS one. 2015;10(12):e0142722. Epub 2015/12/08. doi: 10.1371/journal.pone.0142722. PubMed PMID: 26642214; PMCID: Pmc4671641
7. Luz PM, Belaunzaran-Zamudio PF, Crabtree-Ramirez B, Caro-Vega Y, Hoces D, Rebeiro PF, Blevins M, Pape JW, Cortes CP, Padgett D, Cahn P, Veloso VG, McGowan CC, Grinsztejn B, Shepherd BE. CD4 Response Up to 5 Years After Combination Antiretroviral Therapy in Human Immunodeficiency Virus-Infected Patients in Latin America and the Caribbean. Open forum infectious diseases. 2015;2(2):ofv079. Epub 2015/07/17. doi: 10.1093/ofid/ofv079. PubMed PMID: 26180829; PMCID: Pmc4498272.
8. Morawski BM, Yunus M, Kerukadho E, et al. Hookworm infection is associated with decreased CD4+ T cell counts in HIV-infected adult Ugandans. PLoS Negl Trop Dis. 2017;11(5):e0005634. Published 2017 May 25. doi:10.1371/journal.pntd.0005634
9. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, Pedersen C, Ruxrungtham K, Lewin SR, Emery S, Neaton JD, Brenchley JM, Deeks SG, Sereti I, Douek DC. Plasma Levels of Soluble CD14 Independently Predict Mortality in HIV Infection. J Infect Dis. 2011. Epub 2011/01/22. doi: jiq118 [pii]10.1093/infdis/jiq118. PubMed PMID: 21252259.
10. Hunt PW, Sinclair E, Rodriguez B, Shive C, Clagett B, Funderburg N, Robinson J, Huang Y, Epling L, Martin JN, Deeks SG, Meinert CL, Van Natta ML, Jabs DA, Lederman MM. Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. J Infect Dis. 2014;210(8):1228-38. doi: 10.1093/infdis/jiu238. PubMed PMID: 24755434; PMCID: PMC4192038.
11. Kelesidis T, Kendall MA, Yang OO, Hodis HN, Currier JS. Biomarkers of microbial translocation and macrophage activation: association with progression of subclinical atherosclerosis in HIV-1 infection. J Infect Dis. 2012;206(10):1558–1567. doi:10.1093/infdis/jis545
12. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis. 2014;210(8):1248–1259. doi:10.1093/infdis/jiu254
13. Longenecker CT, Jiang Y, Orringer CE, et al. Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection. AIDS. 2014;28(7):969–977. doi:10.1097/QAD.0000000000000158
14. Knudsen T, Ertner G, Petersen J, et al. Plasma Soluble CD163 Level Independently Predicts All-Cause Mortality in HIV-1–Infected Individuals, The Journal of Infectious Diseases, Volume 214, Issue 8, 15 October 2016, Pages 1198–1204, https://doi.org/10.1093/infdis/jiw263
15. Reimer-McAtee MJ, Somadunderam A, Xu T, Arduino R; Serpa J, Mejia R, Utay N (2019). Effects of Parasitic Infections on Gut Epithelial Barrier and Immune Activation among Foreign-Born HIV-Infected

Patients. Poster presented at Infectious Diseases Society of America, ID Week, Washington DC.
16. George PJ, Anuradha R, Kumar NP, Kumaraswami V, Nutman TB, Babu S. Evidence of microbial translocation associated with perturbations in T cell and antigen-presenting cell homeostasis in hookworm infections. PLoS neglected tropical diseases. 2012;6(10):e1830. Epub 2012/10/12. doi: 10.1371/journal.pntd.0001830. PubMed PMID: 23056659; PMCID: Pmc3464301.
17. Mejia R, Vicuña Y, Broncano N, et al. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. Am J Trop Med Hyg. 2013;88(6):1041–1047. doi:10.4269/ajtmh.12-0726
18. Cimino RO, Jeun R, Juarez M, Cajal PS, Vargas P, Echazu A, Bryan PE, Nasser J, Krolewiecki A, Mejia R. Identification of human intestinal parasites affecting an asymptomatic peri-urban Argentinian population using multi-parallel quantitative real-time polymerase chain reaction. Parasites & vectors. 2015;8:380. Epub 2015/07/18. doi: 10.1186/s13071-015-0994-z. PubMed PMID: 26183074; PMCID: Pmc4504406.
19. Assefa, S., Erko, B., Medhin, G. *et al.* Intestinal parasitic infections in relation to HIV/AIDS status, diarrhea and CD4 T-cell count. *BMC Infect Dis* **9,** 155 (2009). https://doi.org/10.1186/1471-2334-9-155
20. Geng EH, Neilands TB, Thiebaut R, Bwana MB, Nash D, Moore RD, Wood R, Zannou DM, Althoff KN, Lim PL, Nachega JB, Easterbrook PJ, Kambugu A, Little F, Nakigozi G, Nakanjako D, Kiggundu V, Ki Li PC, Bangsberg DR, Fox MP, Prozesky H, Hunt PW, Davies MA, Reynolds SJ, Egger M, Yiannoutsos CT, Vittinghoff EV, Deeks SG, Martin JN. CD41 T cell recovery during suppression of HIV replication: an international comparison of the immunological efficacy of antiretroviral therapy in North America, Asia and Africa. International journal of epidemiology. 2015;44(1):251-63. Epub 2015/04/11. PubMed PMID: 25859596; PMCID: Pmc4339766.
21. Nash D, Katyal M, Brinkhof MW, Keiser O, May M, Hughes R, Dabis F, Wood R, Sprinz E, Schechter M, Egger M. Long-term immunologic response to antiretroviral therapy in low-income countries: a collaborative analysis of prospective studies. AIDS (London, England). 2008;22(17):2291-302. Epub 2008/11/05. doi: 10.1097/QAD.0b013e3283121ca9. PubMed PMID: 18981768; PMCID: Pmc2794130.
22. Yazdanbakhsh M, van den Biggelaar A, Maizels RM. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends in immunology. 2001;22(7):372-7. Epub 2001/06/29. PubMed PMID: 11429321.
23. Maizels RM, Bundy DA, Selkirk ME, Smith DF, Anderson RM. Immunological modulation and evasion by helminth parasites in human populations. Nature. 1993;365(6449):797-805. Epub 1993/10/28. doi: 10.1038/365797a0. PubMed PMID: 8413664.
24. Borkow G, Leng Q, Weisman Z, Stein M, Galai N, Kalinkovich A, Bentwich Z. Chronic immune activation associated with intestinal helminth infections results in impaired signal transduction and anergy. The Journal of clinical investigation. 2000;106(8):1053-60. Epub 2000/10/18. doi: 10.1172/jci10182. PubMed PMID: 11032865; PMCID: Pmc314342.
25. Gallin M, Edmonds K, Ellner JJ, Erttmann KD, White AT, Newland HS, Taylor HR, Greene BM. Cell-mediated immune responses in human infection with Onchocerca volvulus. Journal of immunology (Baltimore, Md : 1950). 1988;140(6):1999-2007. Epub 1988/03/15. PubMed PMID: 3257994.
26. Sher A, Fiorentino D, Caspar P, Pearce E, Mosmann T. Production of IL-10 by CD4+ T lymphocytes correlates with down-regulation of Th1 cytokine synthesis in helminth infection. Journal of immunology (Baltimore, Md : 1950). 1991;147(8):2713-6. Epub 1991/10/15. PubMed PMID: 1680917.
27. Osborne LC, Monticelli LA, Nice TJ, Sutherland TE, Siracusa MC, Hepworth MR, Tomov VT, Kobuley D, Tran SV, Bittinger K, Bailey AG, Laughlin AL, Boucher JL, Wherry EJ, Bushman FD, Allen JE, Virgin HW, Artis D. Coinfection. Virus-helminth coinfection reveals a microbiota-independent mechanism of immunomodulation. Science (New York, NY). 2014;345(6196):578-82. Epub 2014/08/02. doi: 10.1126/science.1256942. PubMed PMID: 25082704; PMCID: Pmc4548887
28. Chachage M, Podola L, Clowes P, Nsojo A, Bauer A, Mgaya O, Kowour D, Froeschl G, Maboko L, Hoelscher M, Saathoff E, Geldmacher C. Helminth-associated systemic immune activation and HIV co-receptor expression: response to albendazole/praziquantel treatment. PLoS neglected tropical diseases. 2014;8(3):e2755. Epub 2014/03/29. doi: 10.1371/journal.pntd.0002755. PubMed PMID: 24675895; PMCID: Pmc3967945.
29. Geiger SM, Caldas IR, Mc Glone BE, Campi-Azevedo AC, De Oliveira LM, Brooker S, Diemert D, Correa-Oliveira R, Bethony JM. Stage-specific immune responses in human Necator americanus

infection. Parasite immunology. 2007;29(7):347-58. Epub 2007/06/20. doi: 10.1111/j.1365-3024.2007.00950.x. PubMed PMID: 17576364; PMCID: Pmc1976388.
30. Kalinkovich A, Weisman Z, Greenberg Z, Nahmias J, Eitan S, Stein M, Bentwich Z. Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. Clinical and experimental immunology. 1998;114(3):414-21. Epub 1998/12/09. PubMed PMID: 9844052; PMCID: Pmc1905129.
31. Gazzinelli-Guimaraes PH, Souza-Fagundes EM, Cancado GG, Martins VG, Dhom-Lemos LC, Ricci ND, Fiuza JA, Bueno LL, Miranda RR, Guatimosim S, Gazzinelli A, Correa-Oliveira R, Bartholomeu DC, Fujiwara RT. Cell apoptosis induced by hookworm antigens: a strategy of immunomodulation. Frontiers in bioscience (Elite edition). 2013;5:662-75. Epub 2013/01/02. PubMed PMID: 23277021.
32. Loukas A, Constant SL, Bethony JM. Immunobiology of hookworm infection. FEMS immunology and medical microbiology. 2005;43(2):115-24. Epub 2005/02/01. doi: 10.1016/j.femsim.2004.11.006. PubMed PMID: 15681140.
33. Johansson ME, Sjovall H, Hansson GC. The gastrointestinal mucus system in health and disease. Nature reviews Gastroenterology & hepatology. 2013;10(6):352-61. Epub 2013/03/13. doi: 10.1038/nrgastro.2013.35. PubMed PMID: 23478383; PMCID: Pmc3758667.
34. Farid AS, Jimi F, Inagaki-Ohara K, Horii Y. Increased intestinal endotoxin absorption during enteric nematode but not protozoal infections through a mast cell-mediated mechanism. Shock (Augusta, Ga). 2008;29(6):709-16. Epub 2007/11/14. doi: 10.1097/shk.0b013e31815c3f36. PubMed PMID: 17998888.
35. George PJ, Anuradha R, Kumar NP, Kumaraswami V, Nutman TB, Babu S. Evidence of microbial translocation associated with perturbations in T cell and antigen-presenting cell homeostasis in hookworm infections. PLoS neglected tropical diseases. 2012;6(10):e1830. Epub 2012/10/12. doi: 10.1371/journal.pntd.0001830. PubMed PMID: 23056659; PMCID: Pmc3464301.
36. Onguru D, Liang Y, Griffith Q, Nikolajczyk B, Mwinzi P, Ganley-Leal L. Human schistosomiasis is associated with endotoxemia and Toll-like receptor 2- and 4-bearing B cells. The American journal of tropical medicine and hygiene. 2011;84(2):321-4. Epub 2011/02/05. doi: 10.4269/ajtmh.2011.10-0397. PubMed PMID: 21292908; PMCID: Pmc3029191.
37. Aksoy E, Zouain CS, Vanhoutte F, Fontaine J, Pavelka N, Thieblemont N, Willems F, Ricciardi-Castagnoli P, Goldman M, Capron M, Ryffel B, Trottein F. Double-stranded RNAs from the helminth parasite Schistosoma activate TLR3 in dendritic cells. The Journal of biological chemistry. 2005;280(1):277-83. Epub 2004/11/03. doi: 10.1074/jbc.M411223200. PubMed PMID: 15519998.
38. Turner JD, Jenkins GR, Hogg KG, Aynsley SA, Paveley RA, Cook PC, Coles MC, Mountford AP. CD4+CD25+ regulatory cells contribute to the regulation of colonic Th2 granulomatous pathology caused by schistosome infection. PLoS neglected tropical diseases. 2011;5(8):e1269. Epub 2011/08/23. doi: 10.1371/journal.pntd.0001269. PubMed PMID: 21858239; PMCID: Pmc3153428.
39. Singh KP, Gerard HC, Hudson AP, Boros DL. Differential expression of collagen, MMP, TIMP and fibrogenic-cytokine genes in the granulomatous colon of Schistosoma mansoni-infected mice. Annals of tropical medicine and parasitology. 2006;100(7):611-20. Epub 2006/09/23. doi: 10.1179/136485906x118530. PubMed PMID: 16989687.
40. Ramakrishnan K, Shenbagarathai R, Uma A, Kavitha K, Rajendran R, Thirumalaikolundusubramanian P. Prevalence of intestinal parasitic infestation in HIV/AIDS patients with diarrhea in Madurai City, South India. Japanese journal of infectious diseases. 2007;60(4):209-10. Epub 2007/07/24. PubMed PMID: 17642535.
41. Tian LG, Wang TP, Lv S, Wang FF, Guo J, Yin XM, Cai YC, Dickey MK, Steinmann P, Chen JX. HIV and intestinal parasite co-infections among a Chinese population: an immunological profile. Infectious diseases of poverty. 2013;2(1):18. Epub 2013/08/27. doi: 10.1186/2049-9957-2-18. PubMed PMID: 23971713; PMCID: Pmc3766051.
42. Arndt MB, John-Stewart G, Richardson BA, Singa B, van Lieshout L, Verweij JJ, Sangare LR, Mbogo LW, Naulikha JM, Walson JL. Impact of helminth diagnostic test performance on estimation of risk factors and outcomes in HIV-positive adults. PloS one. 2013;8(12):e81915. Epub 2013/12/11. doi: 10.1371/journal.pone.0081915. PubMed PMID: 24324729; PMCID: Pmc3852669.
43. Walson JL, Stewart BT, Sangare L, Mbogo LW, Otieno PA, Piper BK, Richardson BA, John-Stewart G. Prevalence and correlates of helminth co-infection in Kenyan HIV-1 infected adults. PLoS neglected

tropical diseases. 2010;4(3):e644. Epub 2010/04/03. doi: 10.1371/journal.pntd.0000644. PubMed PMID: 20361031; PMCID: Pmc2846937.
44. Webb EL, Ekii AO, Pala P. Epidemiology and immunology of helminth-HIV interactions. Current opinion in HIV and AIDS. 2012;7(3):245-53. Epub 2012/03/14. doi: 10.1097/COH.0b013e32835210cd. PubMed PMID: 22411451.
45. Ivan E, Crowther NJ, Mutimura E, Rucogoza A, Janssen S, Njunwa KK, Grobusch MP. Effect of deworming on disease progression markers in HIV-1-infected pregnant women on antiretroviral therapy: a longitudinal observational study from Rwanda. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;60(1):135-42. Epub 2014/09/12. doi: 10.1093/cid/ciu715. PubMed PMID: 25210019.
46. Abossie A, Petros B. Deworming and the immune status of HIV positive pre-antiretroviral therapy individuals in Arba Minch, Chencha and Gidole hospitals, Southern Ethiopia. BMC research notes. 2015;8:483. Epub 2015/09/30. doi: 10.1186/s13104-015-1461-9. PubMed PMID: 26415705; PMCID: Pmc4585997
47. Walson JL, Otieno PA, Mbuchi M, Richardson BA, Lohman-Payne B, Macharia SW, Overbaugh J, Berkley J, Sanders EJ, Chung MH, John-Stewart GC. Albendazole treatment of HIV-1 and helminth co-infection: a randomized, double-blind, placebo-controlled trial. AIDS (London, England). 2008;22(13):1601-9. Epub 2008/08/02. doi: 10.1097/QAD.0b013e32830a502e. PubMed PMID: 18670219; PMCID: Pmc2637615.
48. Means AR, Burns P, Sinclair D, Walson JL. Antihelminthics in helminth-endemic areas: effects on HIV disease progression. Cochrane Database Syst Rev. 2016 Apr 14;4(4):CD006419. doi: 10.1002/14651858.CD006419.pub4. PMID: 27075622; PMCID: PMC4963621.
49. Lankowski AJ, Tsai AC, Kanyesigye M, Bwana M, Haberer JE, Wenger M, Martin JN, Bangsberg DR, Hunt PW, Siedner MJ. Empiric deworming and CD4 count recovery in HIV-infected Ugandans initiating antiretroviral therapy. PLoS neglected tropical diseases. 2014;8(8):e3036. Epub 2014/08/08. doi: 10.1371/journal.pntd.0003036. PubMed PMID: 25101890; PMCID: Pmc4125278.
50. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L, Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12(12):1365-71. PubMed PMID: 17115046.
51. Ericsen AJ, Lauck M, Mohns MS, DiNapoli SR, Mutschler JP, Greene JM, Burwitz BJ, Sacha JB, Friedrich TC, Brenchley JM, O'Connor DH. Hyperacute Microbial Translocation During Pathogenic SIV Infection. Conference on Retroviruses and Opportunistic Infections. 2016;21.
52. Funderburg NT, Mayne E, Sieg SF, Asaad R, Jiang W, Kalinowska M, Luciano AA, Stevens W, Rodriguez B, Brenchley JM, Douek DC, Lederman MM. Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. Blood. 2010;115(2):161-7. Epub 2009/10/16. doi: 10.1182/blood-2009-03-210179. PubMed PMID: 19828697; PMCID: Pmc2808148.
53. Estes JD, Harris LD, Klatt NR, Tabb B, Pittaluga S, Paiardini M, Barclay GR, Smedley J, Pung R, Oliveira KM, Hirsch VM, Silvestri G, Douek DC, Miller CJ, Haase AT, Lifson J, Brenchley JM. Damaged intestinal epithelial integrity linked to microbial translocation in pathogenic simian immunodeficiency virus infections. PLoS pathogens. 2010;6(8):e1001052. Epub 2010/09/03. doi: 10.1371/journal.ppat.1001052. PubMed PMID: 20808901; PMCID: Pmc2924359.
54. Pandrea I, Xu C, Stock JL, Frank DN, Ma D, Policicchio BB, He T, Kristoff J, Cornell E, Haret-Richter GS, Trichel A, Ribeiro RM, Tracy R, Wilson C, Landay AL, Apetrei C. Antibiotic and Antiinflammatory Therapy Transiently Reduces Inflammation and Hypercoagulation in Acutely SIV-Infected Pigtailed Macaques. PLoS pathogens. 2016;12(1):e1005384. Epub 2016/01/15. doi: 10.1371/journal.ppat.1005384. PubMed PMID: 26764484; PMCID: Pmc4713071.
55. George PJ, Anuradha R, Kumar NP, Kumaraswami V, Nutman TB, Babu S. Evidence of microbial translocation associated with perturbations in T cell and antigen-presenting cell homeostasis in hookworm infections. PLoS neglected tropical diseases. 2012;6(10):e1830. Epub 2012/10/12. doi: 10.1371/journal.pntd.0001830. PubMed PMID: 23056659; PMCID: Pmc3464301.

56. Bernard J C Macatangay, Edwin K Jackson, Kaleab Z Abebe, et al. A Randomized, Placebo-Controlled, Pilot Clinical Trial of Dipyridamole to Decrease Human Immunodeficiency Virus–Associated Chronic Inflammation, The Journal of Infectious Diseases, , jiz344, https://doi.org/10.1093/infdis/jiz344
57. Tae Joon Yi, Sharon Walmsley, Leah Szadkowski, et al. A Randomized Controlled Pilot Trial of Valacyclovir for Attenuating Inflammation and Immune Activation in HIV/Herpes Simplex Virus 2–Coinfected Adults on Suppressive Antiretroviral Therapy, Clinical Infectious Diseases, Volume 57, Issue 9, 1 November 2013, Pages 1331–1338, https://doi.org/10.1093/cid/cit539
58. Kostadinova L, Shive CL, Zebrowski E, et al. Soluble Markers of Immune Activation Differentially Normalize and Selectively Associate with Improvement in AST, ALT, Albumin, and Transient Elastography During IFN-Free HCV Therapy. Pathog Immun. 2018;3(1):149–163. doi:10.20411/pai.v3i1.242
59. Cheru LT, Park EA, Saylor CF, Burdo TH, Fitch KV, Looby S, Weiner J, Robinson JA, Hubbard J, Torriani M, Lo J. I-FABP Is Higher in People With Chronic HIV Than Elite Controllers, Related to Sugar and Fatty Acid Intake and Inversely Related to Body Fat in People With HIV. Open Forum Infect Dis. 2018 Nov 5;5(11):ofy288. doi: 10.1093/ofid/ofy288. PMID: 30515430; PMCID: PMC6262112.
60. O'Keeffe AG, Ambler G, Barber JA. Sample size calculations based on a difference in medians for positively skewed outcomes in health care studies. BMC Med Res Methodol. 2017 Dec 2;17(1):157. doi: 10.1186/s12874-017-0426-1. PMID: 29197347; PMCID: PMC5712177.