**Concept Sheet *Research Plan* Form  
MACS/WIHS Combined Cohort Study (CCS)**[**MWCCS@jhu.edu**](mailto:MWCCS@jhu.edu)

Please collaborate with your co-investigators, site-PI, or CCS liaison in the construction of your Research Plan using the following template. You may insert relevant figures, tables or images into this template. Once completed, upload this document as a single file to your online concept submission form as part of Section C.

1. **Concept Sheet Title**

HIV influenced metabolomic signatures and HIV-related chronic comorbidities related traits.

1. **Abstract (Maximum 400 words)**

**Backgrounds:**

Combination Antiretroviral treatments (ART) use improves health, prolongs life, and substantially reduces the risk of HIV transmission. {Deeks, 2013 #365} However, these treatments does not fully restore health. {Deeks, 2009 #366;Deeks, 2013 #365} Also, HIV-infected patients has higher risk of variable non-aids chronical disease, which were independent from aging and AIDS status, in PLWH than non-HIV-infected person. {Negin, 2012 #362} Notably, in lipid metabolism, ART use changed dramatically in human body and it occurred metabolic complications. {Nakaranurack, 2018 #364} {Trevillyan, 2018 #127} it changed the plasma metabolites level.

Previous research provide the association between HIV-infection altered inflammation, other disease risk factors, and immune activation markers. {Hanna, 2017 #57} also, these alteration affected to various chronic disease (e.g., CVD, diabetes, obesity, Hypertension, dyslipidemia, cancer, kidney failure, liver failure, and neurocognitive decline). For example, specific risk factors associated with increased risk of chronic disease. {Kaplan, 2016 #72;Hanna, 2015 #64} metabolites can use as biomarker for predicted these chronic disease.

However, previous comorbidities studies focused on the disease and basic sociodemographic (e.g., age, sex, CD4 level?), not a disease related factors. {} disease based approach has limitation. Some cohort study cannot detect some disease? And/or if we use disease related traits, it will be helpful to predict traits related disease before the developed chronic disease. Also, it will be helpful to predicted correlation between major disease traits and disease, which shred several disease risk factors (e.g., blood pressure, traditional lipids measurements). using the metabolomic profiles is final product of biological process. so if we use this metabolites profiles as biomarkers.

Other cohort study about comorbidities {Schouten, 2014 #367}

cross-sectional analysis of comorbidities in Canadian HIV-infected population.{Kendall, 2014 #371}

other confounding factors?

chronic comorbidities, which may be due to chronic inflammation from long-term HIV infection as well as ART toxicities exacerbated by aging.{Negin, 2012 #362}

the prevalence of non-aids comorbidities, particularly metabolomic complications is high in HIV-infected individuals.

**Purpose:**

we can identified chronic comorbidities associated metabolites through the disease related risk factors and traits with bioinformatical analysis approaches. And we provide insight or big pictures of metabolic signatures influenced by HIV status and related comorbidities related traits. Also, these metabolic signature should be used to new biomarker for pre-non-AIDS comorbidities. Ans this finding offered HIV-infected patients living like non-infected person without non-aids comorbidities with correct or efficient HIV treatment and care.

The purpose of this research is to advance knowledge of the HIV infection, with a focus on HIV-related comorbidities, by the Multi-Center AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS) Combined Cohort Study (MACS/WIHS-CCS).

**Methods**:

We profiled 322 plasma metabolites profiles from lipidomic platform and polar metabolites platform in 737 participants aged 35-55 years (520 HIV+, 217 HIV-) from two prospective cohorts: the Women’s Interagency HIV Study (WIHS) and the Multicenter AIDS Cohort Study (MACS). Linear regression models was used to examine the HIV status influenced metabolite profiles , associations of baseline HIV status with metabolites profiles measures. The least absolute shrinkage and selection operator (LASSO) was used for metabolites selection in the setting of high data dimensionality and extensive intercorrelations. We implements partial spearman correlation for estimated association between metabolites signatures and comorbidities related factors.

**Results:**

Afteradjusting for demographic and behavioral factors, we identified 36 metabolites from HILIC platform and XX lipid species form lipidomic platform after linear regression, that influenced by HIV status. (all *P*<0.05 after multiple testing correction). Total of 21 metabolites and 16 lipid species remained significant after LASSO for metabolites selection.

Further significantly associated with HIV-status. We compared these selected significant metabolites with disease related risk factors (continuous variables only). although many of them showed moderate-to-high correlations with conventional blood lipids (e.g. total-cholesterol, LDL-cholesterol, triglycerides). We identified XX metabolites associated with diabetes risk factors (e.g., glucose, insulin, and BMI?) also, we defined these metabolites associated with increased risk of diabetes. Or interpretation of other disease related metabolites.

~~Ex) XXXX, XXXX and XXXX, respectively, showed the strongest associations with increased risk of carotid artery plaque. All the results were generally consistent between WIHS women and MACS men.~~

**Conclusion**

We identified association of metabolic signature complications with HIV-related chronic comorbidities in PLWH. the finding of this study may help us to better understand association of metabolomic signatures with disease related factors in PLWH and find way to lower their risk of chronic HIV-related comorbidities. Our finding provide new guide line for improve health condition and quality of life in HIV-infected patients. therefore, a comprehensive approached to managing such complications should be used when treating HIV-infected participants to achieve long-term survival. {Kendall, 2014 #371} Also, these significant metabolites signatures help additional pharmacovigilance of ART use.

**keywords**

People living with HIV, Antiretroviral Treatment use, HIV-related chronic comorbidities, Metabolomic signatures

1. **Background (Maximum 400 words, not including references)**

**Backgrounds:**

People with HIV infection can achieve nearly normal life-spans if treated with effective ART, in which case they are more likely to suffer chronic HIV-related comorbidities than AIDS-defining conditions.

Combination Antiretroviral treatments (ART) use improves health, prolongs life, and substantially reduces the risk of HIV transmission. {Deeks, 2013 #365} However, these treatments does not fully restore health. {Deeks, 2009 #366;Deeks, 2013 #365} Also, HIV-infected patients has higher risk of variable non-aids chronical disease, which were independent from aging and AIDS status, in PLWH than non-HIV-infected person. {Negin, 2012 #362} Notably, in lipid metabolism, ART use changed dramatically in human body and it occurred metabolic complications. {Nakaranurack, 2018 #364} {Trevillyan, 2018 #127} it changed the plasma metabolites level. And it influenced altered metabolites levels in human body. {Zhao, 2019 #380} integration of these non-aids chronic diseases become a chronic comorbidities.

Previous research provide the association between HIV-infection altered inflammation, other disease risk factors, and immune activation markers. {Hanna, 2017 #86} also HIV-infected patients showed different metabolites levels than non-infected individuals. {Qi, 2018 #350} These inflammation and immune activation marker influenced to development other chronic diseases. (liver) {Chen, 2015 #381}

also, these metabolic alteration should be affected to various chronic disease (e.g., CVD{Kaplan, 2016 #72;Hanna, 2015 #64;Dau, 2008 #379}, diabetes{}, obesity{}, Hypertension{}, dyslipidemia{}, cancer{}, kidney failure{}, liver failure{}, and neurocognitive decline{}).

For example, specific risk factors associated with increased risk of chronic disease. {Kaplan, 2016 #72} metabolites can use as biomarker for predicted these chronic disease in individual study. However in the chronic disease, their connectivity is very high between each other’s.

And these HIV-related comorbidities developments to mortality? {Hanna, 2018 #354}

However, previous comorbidities studies focused on the disease outcome and basic sociodemographic (e.g., age, sex, CD4 level, HIV viral load), not a disease related factors. {??} Disease based approach has limitation. many chronic disease associations each other’s and many of them shared major risk factors (e.g., bp, traditional lipids measurement).

Some cohort study cannot detect some disease? And/or if we use disease related traits, it will be helpful to predict traits related disease before the developed chronic disease. Also, it will be helpful to predicted correlation between major disease traits and disease, which shred several disease risk factors (e.g., blood pressure, traditional lipids measurements). using the metabolomic profiles is final product of biological process. so if we use this metabolites profiles as biomarkers.

Metabolites can use as biomarker . some lipid species increased risk of CVD {Zhao, 2019 #380} and it highly correlated with traditional lipids measurements.

Other cohort study about comorbidities {Schouten, 2014 #367}

cross-sectional analysis of comorbidities in Canadian HIV-infected population.{Kendall, 2014 #371}

**Purpose:**

we can identified chronic comorbidities associated metabolites through the disease related risk factors and traits with bioinformatical analysis approaches.

And we provide insight or big pictures of metabolic signatures influenced by HIV status and related comorbidities related traits. Also, these metabolic signature should be used to new biomarker for pre-non-AIDS comorbidities. Ans this finding offered HIV-infected patients living like non-infected person without non-aids comorbidities with correct or efficient HIV treatment and care.

The purpose of this research is to advance knowledge of the HIV infection, with a focus on HIV-related comorbidities, by the Multi-Center AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS) Combined Cohort Study (MACS/WIHS-CCS).

\_\_ need organization

Especially, metabolomics influenced by genetic variation and environments factors (e.g., food intake and drug use and physical activity). And HIV-infection altered metabolic levels in the body and this works as new biomarker for HIV-infected participants. Furthermore this metabolites signatures use as other chronic disease biomarkers. {}

Is it possible to fine novel causal marker or factor by the mendelian randomization?

How can I finished or summarized this study?

HIV-infection caused metabolic abnormalities in PLWH and this abnormality change the lipid metabolism and glycolytic metabolism.

non-AIDS comorbidities bas become a major concern in PLWH receiving ART treatment, yet an understanding of its pathophysiology remains incompletes and influence of chronic disease related factors.

Life expectancy in people living with HIV (PLWH) has increased in the past decades, since the introduction of highly active antiretroviral treatment (ART). {Peterson, 2019 #361}

The success of antiretroviral therapy (ART) has ushered in a new era of clinical management for HIV patients. Today almost half of HIV infected patients in North America are over 50 years old. People with HIV infection can achieve nearly normal life-spans if treated with effective ART, in which case they are more likely to suffer chronic HIV-related comorbidities than AIDS-defining conditions. Despite this success, the impact of HIV, its treatment, and the legacy of immune suppression needs to be understood to optimize the health of people living with HIV.

Metabolites were end product from biochemical synthesis in human body, which can be used as biomarker for dysmetabolic disease like diabetes, obesity, and cardiovascular disease.[] HIV infection related factors like ART toxicity, drug–drug interactions, immune dysregulation and chronic inflammation should be changed the PLWH metabolism.

Life expectancy in people living with HIV (PLWH) has increased in the past decades, since the introduction of highly active antiretroviral treatment (ART). {Peterson, 2019 #361} Also, biomarkers of monocyte and macrophage activation (e.g. IL-6, sCD14, sCD163, hsCRP, sTNFR-1, and sTNFR-2) have shown the most consistent effects across previous studies [David’s, 21-25]. These inflammation markers influences several chronic diseases such as cardiovascular disease, cancer, lung disease, metabolic disease, neurocognitive disorder, low bone mineral density, and frailty.[]

The older HIV patient poses a unique challenge, as management should take into account different factors, some related to global ageing such as geriatric syndromes, traditional risk factors, social vulnerability, and age-related diseases, and others related to HIV infection like ART toxicity, drug–drug interactions, immune dysregulation and chronic inflammation. All the above can amount to great polypharmacy and multimorbidity that physician have to be aware of. Little is known about the best screening, management and treatment strategies to improve long-term health outcomes in this ageing population. The following article briefly reviews the main comorbidities that can affect the ageing HIV patient.

The scale-up of antiretroviral therapy has significantly reduced the devastating impact of the global HIV epidemic in recent decade. HIV infection has become a chronic disease, and people living with HIV (PLHIV) are now surviving, ageing, and requiring lifelong care and treatment. Across all age groups, PLHIV have an increased risk of chronic complications and comorbidities, such as noncommunicable diseases and mental, neurological and substance-use disorders. These conditions may be pre-existing, HIV-associated or due to ageing.

Other cohort study about comorbidities {Schouten, 2014 #367}

cross-sectional analysis of comorbidities in Canadian HIV-infected population.{Kendall, 2014 #371}

other confounding factors?

chronic comorbidities, which may be due to chronic inflammation from long-term HIV infection as well as ART toxicities exacerbated by aging.{Negin, 2012 #362}

the prevalence of non-aids comorbidities, particularly metabolomic complications is high in HIV-infected individuals.

1. **Specific Aims & Hypotheses (Maximum 200 words, not including references)**

**1) To identify metabolites signature influenced by HIV-serostatus in people living with HIV (PLWH).**

Using metabolomic analysis, we will identify and characterize the metabolic signature influenced by HIV-status. We will implementing the least absolute shrinkage and selection operator (LASSO) algorithm for the reduce the data dimension and avoid extensive intercorrelations. ~~After implements LASSO, we will characterize association of metabolomic signatures in 6 metabolic super pathway (e.g., Amino acids, Lipids).~~ We will compare identified metabolic signatures in HIV+ and HIV- individuals with ART use. ***We hypothesize*** that certain HIV infection contribute to altered metabolic signatures, by affecting the transcriptional network of innate immune cells and lipid metabolism and ART use.

**2) To identify metabolic signatures and related pathways associated with comorbidities related factors in PLWH.**

relationship with significant metabolites and comorbidities related variables.

most of significant metabolites should be associated with several disease related risk factors.

***We hypothesize*** that HIV-infection influenced metabolite signatures represents association with major metabolic pathway (in this case I can see the host of significant metabolites related with Arginine metabolism. And some lipid metabolism.)

**3) To estimate the role of metabolic signatures in the progression of chronic comorbidities in HIV infected individuals.**

Longitudinal analyses will examine metabolites, comorbidities related factors. We will provide overview of the network of comorbidities related factors by metabolic signatures.

***We hypothesize that*** HIV-influenced metabolic signatures,

and association with comorbidities risk factors and there correlation that results from these metabolic signatures,

contributes to progression of chronic comorbidities, which may explain in part the increased chronic comorbidities risk in HIV-infected individuals.

1. **Approach**Please include a summarized study design(s), inclusion/exclusion criteria, analytical methods, & sample size calculations.

Also we collected disease related continuous risk factors (e.g., BP, etc.). explain how to collect data from MACS/WIHS cohort study.

linear regression model adjusted for age, sex, race, center, smoking, HCV status.

Partial Spearman correlation.

LASSO for metabolites selection

GGM for interaction? network modules?

**Study design**

This proposed project will be conducted among WIHS and MACS cohort studies.

collect disease related variables

The metabolic comorbidities in the study included hyperlipidemia, hypertension, diabetes mellitus, and impaired fasting glucose. Viral hepatitis coinfections included hepatitis B and hepatitis C. Neurological diseases included stroke, Parkinson disease, epilepsy, and dementia. Cardiovascular diseases included congestive heart failure, myocardial infarction, and cardiomyopathy. Thyroid diseases included hyperthyroid, hypothyroid, thyrotoxicosis, and nontoxic thyroid nodules. {Nakaranurack, 2018 #364}

the metabolic comorbidities in the study include blood related factors (e.g., SBP, DBP, ... ), cardiovascular disease related factor (), Medical Risk Factor (), Sociodemographic (), Neurocognitive (), lipid related factors (), and HIV related Lab measurements ().

we get 322 known metabolites form two metabolomics platform

**HILIC-positive platform:** Amines and polar metabolites that ionize in the positive ion mode using hydrophilic interaction liquid chromatography (HILIC) and MS analyses

HILIC-negative platform: Central metabolites and polar metabolites that ionize in the negative ion mode using HILIC chromatography with an amine column and targeted MS

**C8** **platform**: Polar and non-polar lipids using reverse phase chromatography and full scan MS

C18 platform: Free fatty acids, bile acids, and metabolites of intermediate polarity using reversed chromatography with a T3 UPLC column (C18 chromatography) and MS analyses in the negative ion mode

Metabolites are then identified by examining their mass-to-charge (m/z) ratio and retention time (rt) on the chromatographic output. The experiment will be spiked with known standards.

**Variables** (Table)

HIV-related disease = including cardiovascular disease, malignancy, renal disease, liver disease, bone disease, and perhaps neurological complications, which are phenomena of the normal aging process.

Hypertension

Diabetes mellitus and insulin resistance

CVD

pulmonary hypertension

cancer

osteopenia and osteoporosis

liver failure

kidney failure

peripheral neuropathy

frailty

cognitive decline and dementia

And these disease related risk factors and medication use.

**Inclusion/exclusion criteria**

Check the exclusion in 737 samples. (HIV+ = 520, HIV- = 217)

Exclude prevalent CVD and diabetes.

During correlation analysis we exclude medication use (e.g., anti-hypertensive medication, lipid-lowering medication use)

Check the other disease related medication use (kidney, liver, neurocognitive function, etc.)

**Analytical methods**

1. get the significant metabolites influenced by HIV status using linear regression adjusted for age, sex, race, center, and HCV flag.
   1. by linear regression models adjusted for age, sex, race, center, smoking, and HCV status.

2. metabolites selection by LASSO (validated with other statistical methods?).

3. get correlation between selected metabolites by platform.

- kind of cross-sectional analysis.

- get correlation heatmap and this will be helpful to interpretation of data.

4. calculated partial spearman correlation for these selected metabolites with continuous disease variables.

5. check the metabolites related pathway (Metaboanalyst Or KEGG, GO) and relationship with chronic HIV-related comorbidities.

**? metabolites QC**

(1) metabolites from HILIC platform and lipid species from C8 platform with ≥20% missing values and >20 %CV will be excluded from the analyses; missing values for the remaining metabolites will be imputed using half of the lowest detected level before transformation.

(2) We will apply rank-based inverse normal transformation to raw concentrations of the sphingolipid species to obtain standardized scores.

(3) Baseline participant characteristics will be described as weighted means (95% CI) for quantitative variables or percentage (95% CI) for categorical variables by HIV serostatus (HIV-infected, non-infected) (Table 1). Group-specific differences will be compared using survey linear regressions for continuous variables and survey logistic regressions for categorical variables; both account for the complex study design.

(4) Correlations between different metabolites and between metabolites and anthropometry parameters (BMI and WHR) and glycemic and lipid traits and HIV-related factors will be calculated, using the weighted Spearman rank correlation coefficients , with adjustment for age and sex. Results will be presented using a heat map (Figure 1).

**metabolites selection**

(5) The primary method employed for metabolites selection was L1-regularized Cox regression, implementing the least absolute shrinkage and selection operator (LASSO) algorithm [2].

use LASSO and OPLS-Da for metabolites selection (supplement table).

(5-2) For validation purposes, two additional selection approaches were carried out – a best subset and a bagged backwards stepwise procedure. AIC (Akaike information criterion)19 minimization as selection criterion

(6) Multivariable-adjusted relative risks and 95% CIs of diabetes will be calculated using the Poisson regression models. One basic model in addition to two exploratory statistical models with adjustment for different covariates will be used (see above). In these analyses, each metabolites will be analyzed continuously based on the transformed values (Table 2). As such, the results could be explained as for each 1 SD increment in individual metabolites.

(7) The associations between sphingolipid species and baseline BMI, WHR, fasting glucose, fasting insulin, HbA1c, HOMA-IR, HOMA-B, LDL-C, HDL-C, TG, and AHEI-2010 score will be examined using survey linear regression models, with adjustment for covariates listed in Model 1 (except for AHEI-2010). Beta, SE and P values will be presented (Table 3).

(8) Due to the multiple comparisons, all P values generated from the above survey Poisson regression and survey linear regression models (#5 and #6) will be adjusted for the False Discovery Rate (FDR) by the Benjamini and Yekutieli procedure.

We will generate the scores by computing the weighted sum of the sphingolipid species included in subclass. Weights are coefficients from a multivariate Poisson regression model that is fit with each sphingolipid species adjusted for covariates included in the model 1 as described above. Then, we created quartiles for each score and examined their relations to risk of T2D in the multivariable Poisson regression models. (Table 4). P values for trend will be calculated by treating median scores for each quartile as continuous variables.

(10) Sensitivity analyses: because some [9], though not other [8] previous studies have shown that the association of sphingolipids with risk of diabetes may vary by the number of carbons or double bonds in the acyl chain carbons, we will perform the following sensitivity analyses according to the total number of carbons and double bonds:

1) RR values derived from the Model 1, Model 2 and Model 3 in Table 2 will be plotted, and the 3 plots will be combined as one figure. (see Figure 2).

2) Beta values derived from the Model 1 in Table 3 will be plotted, and the 11 plots will be combined in one figure.

**Sample size calculations**

Participants of the WIHS and MACS (N = 737, WIHS = 398, MACS = 339)

**Inclusion/exclusion criteria**

Check the exclusion in 737 samples. (HIV+ = 520, HIV- = 217)

**Analytical methods**

(1) metabolites and lipid species with ≥20% missing values will be excluded from the analyses; missing values for the remaining metabolites will be imputed using half of the lowest detected level before transformation.

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1. **Description of Participant or Site Staff Burden (Maximum 400 words)**For participant burden please provide details if your project will require additional specimen collection, a new questionnaire, new procedures, new informed consent, and/or if your project will require an additional visit. For CCS staff burden please provide details if your project will require IRB submission, staff training, and/or coordinating/consenting /providing participant incentives.
2. **Specimen Characteristics and Laboratory Methods**For projects that will use participant specimens, describe the criteria for selecting specimens, the lab testing methods, and the procedures for QA/QC.
3. **References**

Table 1. basic characteristics

Table . plasma metabolites level in HIV specific variables

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | HIV status | hivtx | cd4\_grp | vload\_det\_grp |
| Metabolites | HIV+ | HIV- | p.val | ART use | non use | p.val | ≤ 500 | > 500 | p.val | undetectable | detectable | p.val |
| No.of.participants | 520/737 | 217/737 |  | 406/520 | 114/520 |  | 273/520 | 247/520 |  | 286/520 | 234/520 |  |
| TAG\_56\_04 | 0.22 | -0.50 | < 0.001 | 0.20 | -0.20 | < 0.001 | 0.08 | 0.21 | 0.099 | 0.19 | 0.08 | 0.215 |
| CE\_22\_04 | 0.21 | -0.09 | < 0.001 | 0.20 | -0.24 | < 0.001 | 0.22 | 0.15 | 0.425 | 0.22 | -0.08 | 0.001 |
| PC\_38\_03 | 0.19 | -0.52 | < 0.001 | 0.23 | -0.56 | < 0.001 | -0.05 | 0.28 | < 0.001 | 0.27 | -0.29 | < 0.001 |
| TAG\_55\_03 | 0.19 | -0.45 | < 0.001 | 0.23 | -0.17 | < 0.001 | 0.15 | 0.24 | 0.287 | 0.23 | 0.11 | 0.197 |
| X5.hydroxytryptophan | 0.13 | -0.63 | < 0.001 | -0.21 | 0.12 | 0.001 | 0.06 | -0.30 | < 0.001 | -0.26 | 0.18 | < 0.001 |
| CER\_16\_00 | 0.11 | -0.47 | < 0.001 | 0.06 | -0.37 | < 0.001 | 0.01 | 0.04 | 0.693 | 0.06 | -0.13 | 0.027 |
| guanine | 0.08 | -0.24 | < 0.001 | -0.07 | -0.43 | 0.001 | -0.03 | -0.13 | 0.244 | -0.08 | -0.20 | 0.164 |
| cytosine | 0.08 | -0.54 | < 0.001 | 0.05 | -0.12 | 0.097 | 0.27 | -0.07 | < 0.001 | 0.01 | 0.19 | 0.036 |
| CE\_22\_05 | 0.05 | -0.13 | 0.023 | 0.05 | -0.21 | 0.023 | 0.04 | 0.03 | 0.893 | 0.06 | -0.13 | 0.039 |
| niacinamide | 0.05 | -0.22 | 0.001 | 0.02 | 0.10 | 0.472 | 0.03 | 0.02 | 0.915 | 0.03 | -0.02 | 0.611 |
| C26.carnitine | 0.04 | -0.43 | < 0.001 | 0.08 | -0.68 | < 0.001 | -0.14 | 0.11 | 0.005 | 0.13 | -0.50 | < 0.001 |
| DMGV | 0.01 | -0.33 | < 0.001 | 0.15 | -0.06 | 0.045 | 0.06 | 0.18 | 0.173 | 0.15 | 0.08 | 0.458 |
| CE\_20\_03 | -0.01 | -0.23 | 0.008 | -0.08 | -0.48 | < 0.001 | -0.16 | -0.07 | 0.315 | -0.04 | -0.41 | < 0.001 |
| N.carbamoyl.beta.alanine | -0.02 | -0.44 | < 0.001 | -0.07 | 0.38 | < 0.001 | 0.17 | -0.14 | 0.001 | -0.11 | 0.32 | < 0.001 |
| ADMA | -0.04 | -0.44 | < 0.001 | -0.14 | 0.05 | 0.067 | -0.04 | -0.17 | 0.154 | -0.16 | 0.03 | 0.041 |
| Pseudouridine | -0.06 | -0.38 | < 0.001 | -0.08 | -0.02 | 0.544 | 0.00 | -0.11 | 0.229 | -0.10 | 0.06 | 0.075 |
| C20.carnitine | -0.07 | -0.39 | < 0.001 | -0.04 | -0.64 | < 0.001 | -0.12 | -0.05 | 0.434 | 0.00 | -0.47 | < 0.001 |
| N.Acetylarginine | -0.07 | -0.31 | 0.002 | -0.08 | -0.14 | 0.621 | -0.05 | -0.11 | 0.535 | -0.10 | -0.03 | 0.450 |
| SM\_18\_02 | -0.07 | 0.26 | < 0.001 | -0.11 | -0.42 | 0.004 | -0.25 | -0.07 | 0.040 | -0.10 | -0.26 | 0.087 |
| C16.carnitine | -0.08 | -0.29 | 0.011 | -0.15 | -0.40 | 0.013 | -0.15 | -0.17 | 0.860 | -0.13 | -0.30 | 0.056 |
| pipecolic.acid | -0.10 | 0.16 | 0.001 | -0.17 | 0.11 | 0.008 | -0.06 | -0.19 | 0.159 | -0.17 | -0.02 | 0.087 |
| Acetaminophen | -0.10 | 0.12 | 0.007 | -0.29 | 0.04 | 0.002 | -0.16 | -0.32 | 0.081 | -0.31 | -0.08 | 0.013 |
| Urocanic.acid | -0.10 | 0.20 | < 0.001 | 0.05 | 0.09 | 0.673 | -0.03 | 0.09 | 0.162 | 0.06 | 0.00 | 0.495 |
| PE\_38\_03\_P | -0.12 | 0.38 | < 0.001 | -0.08 | -0.24 | 0.151 | -0.20 | -0.05 | 0.087 | -0.07 | -0.21 | 0.127 |
| CE\_18\_03 | -0.15 | 0.13 | 0.001 | -0.29 | -0.27 | 0.896 | -0.33 | -0.27 | 0.447 | -0.27 | -0.38 | 0.226 |
| PC\_34\_03\_P | -0.16 | 0.34 | < 0.001 | -0.19 | -0.21 | 0.849 | -0.32 | -0.14 | 0.033 | -0.17 | -0.31 | 0.120 |
| creatinine | -0.20 | 0.06 | 0.001 | -0.25 | 0.06 | 0.005 | -0.08 | -0.30 | 0.021 | -0.28 | 0.02 | 0.002 |
| SM\_24\_00 | -0.20 | 0.23 | < 0.001 | -0.20 | -0.50 | 0.004 | -0.44 | -0.11 | < 0.001 | -0.16 | -0.49 | < 0.001 |
| C20.4.carnitine | -0.21 | 0.20 | < 0.001 | -0.29 | 0.31 | < 0.001 | -0.12 | -0.31 | 0.029 | -0.32 | 0.11 | < 0.001 |
| SM\_22\_01 | -0.23 | 0.13 | < 0.001 | -0.30 | -0.72 | < 0.001 | -0.52 | -0.24 | 0.002 | -0.27 | -0.62 | < 0.001 |
| Ribothymidine | -0.23 | 0.21 | < 0.001 | -0.30 | -0.84 | < 0.001 | -0.60 | -0.21 | < 0.001 | -0.24 | -0.81 | < 0.001 |
| TAG\_54\_10 | -0.24 | 0.17 | < 0.001 | -0.27 | -0.69 | < 0.001 | -0.52 | -0.20 | < 0.001 | -0.25 | -0.55 | 0.001 |
| C8.carnitine | -0.25 | 0.04 | < 0.001 | -0.36 | -0.43 | 0.504 | -0.43 | -0.34 | 0.253 | -0.36 | -0.42 | 0.454 |
| PC\_34\_02\_P | -0.27 | 0.19 | < 0.001 | -0.36 | -0.32 | 0.706 | -0.45 | -0.31 | 0.101 | -0.33 | -0.46 | 0.145 |
| PC\_36\_01\_P | -0.27 | 0.14 | < 0.001 | -0.44 | -0.34 | 0.300 | -0.49 | -0.41 | 0.389 | -0.44 | -0.43 | 0.884 |
| tryptophan | -0.27 | 0.47 | < 0.001 | -0.32 | -0.35 | 0.774 | -0.39 | -0.29 | 0.251 | -0.32 | -0.33 | 0.952 |
| citrulline | -0.39 | -0.10 | < 0.001 | -0.53 | -0.61 | 0.402 | -0.54 | -0.53 | 0.891 | -0.52 | -0.60 | 0.367 |

**Table . disease related continuous variables.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| FORM | NAME | LABEL | V20 | V21 | related disease |
| CVOUTCOME | AVEGSM1 | Grey Scale Median (GSM), only measure or smaller of two | 933 | 582 | CVD |
| CVOUTCOME | AVEGSM2 | Grey Scale Median (GSM), larger of two measures | 5 | 1 | CVD |
| CVOUTCOME | BBPDIAS | MEAN BRACHIAL BLOOD PRESSURE:DIASTOLIC | 0 | 342 | CVD |
| CVOUTCOME | BBPSYS | MEAN BRACHIAL BLOOD PRESSURE:SYSTOLIC | 0 | 342 | CVD |
| CVOUTCOME | BSA | BODY SURFACE AREA: SQRT(Height\*Weight/36) | 0 | 337 | CVD |
| CVOUTCOME | CRP\_W050 | Highly sensitive CRP result from W05015 | 1753 | 674 | inflammation/immune |
| CVOUTCOME | CRP\_W080 | Highly sensitive CRP result from W08034 | 45 | 27 | inflammation/immune |
| CVOUTCOME | CVUSDIAS | Average diastolic blood pressure (from up to 5 readings) | 1144 | 697 | CVD |
| CVOUTCOME | CVUSSYS | Average systolic blood pressure (from up to 5 readings) | 1144 | 697 | CVD |
| CVOUTCOME | DDIMER | D-dimer result | 45 | 27 | inflammation/immune |
| CVOUTCOME | DIST1 | Distensibility index (mmHg) [calculated for waves 1-3 only; need MINCAD & MAXCAD] | 1144 | 697 | CVD |
| CVOUTCOME | DIST2 | Distensibility index (10^-6\*N^-1\*m^2) [calculated for waves 1-3 only; need MINCAD & MAXCAD] | 1144 | 697 | CVD |
| CVOUTCOME | ELAS1 | Young's modulus of elasticity (mmHg) [calculated for waves 1-3 only; need MINCAD & MAXCAD] | 1144 | 697 | CVD |
| CVOUTCOME | ELAS2 | Young's modulus of elasticity (10^5\*N\*m^-2) [calculated for waves 1-3 only; need MINCAD &MAXCAD] | 1144 | 697 | CVD |
| CVOUTCOME | HRTRATE | Average resting heart rate (from up to 5 readings) | 1142 | 697 | Blood |
| CVOUTCOME | IL6 | IL-6 result | 45 | 27 | inflammation/immune |
| CVOUTCOME | IMT | Right common carotid irtery IMT (mm) | 1144 | 697 | CVD |
| CVOUTCOME | LVMASS | LEFT VENTRICLE MASS: (((swt+pwt+lveddd)\*\*3-lveddd\*\*3)\*1.04)\*0.8-0.6 | 0 | 342 | CVD |
| CVOUTCOME | LVMSIDX | LV MASS INDEX:LVMASS/BSA | 0 | 337 | CVD |
| CVOUTCOME | MAXCAD | Right common carotid artery maximum diameter (mm) [measured at waves 1-3 only] | 1144 | 697 | CVD |
| CVOUTCOME | MINCAD | Right common carotid artery minimum diameter (mm) [measured at waves 1-3 only] | 1144 | 697 | CVD |
| HEPSUM | HBVDNAB | HBV DNA load at baseline (international units) (1st 3 visits) | 103 | 57 | Liver |
| HEPSUM | HCVRNAB | HCV RNA load at baseline (international units)(1st 3 visits) | 103 | 57 | Liver |
| LABSUM | CD3N | # of CD3 positive cells (cells per mm^3) | 2118 | 1457 | HIV |
| LABSUM | CD4N | # of CD4 positive cells (helpers, cells per mm^3) | 2137 | 1463 | HIV |
| LABSUM | CD8N | # of CD8 positive cells (suppressors, cells per mm^3) | 2137 | 1464 | HIV |
| LABSUM | HSRAT | Helper/Suppressor Ratio (CD4N/CD8N) | 2137 | 1463 | HIV |
| LABSUM | VLOAD | HIV RNA (copies per ml) | 1558 | 1486 | HIV |
| LIPIDS | CHOL | Cholesterol (MG/DL) | 2047 | 2040 | lipids |
| LIPIDS | GLUCS\_P | Glucose, Serum or Plasma (mg/dL) | 1515 | 1539 | lipids |
| LIPIDS | HDLCHOL | HDL Cholesterol (MG/DL) | 2033 | 2034 | lipids |
| LIPIDS | HDLCHOLP | Percent HDL/Cholesterol (%) | 1937 | 1504 | lipids |
| LIPIDS | HEMOGLOB | Hemoglobin A1C (%) | 1932 | 1951 | lipids |
| LIPIDS | INSSERUM | Insulin, Serum (uIU/mL) | 1502 | 1527 | lipids |
| LIPIDS | LDLCHOLC | LDL Cholesterol, Calculated (MG/DL) | 1971 | 1989 | lipids |
| LIPIDS | LDLCHOLD | LDL Cholesterol, Direct (MG/DL) | 1956 | 1994 | lipids |
| LIPIDS | TRIGLYC | Triglycerides (MG/DL) | 2047 | 2040 | lipids |
| LIPIDS | VLDLCHOL | VLDL Cholesterol, Calculated (MG/DL) | 1889 | 1971 | lipids |
| NEUROCOG | DSYM | Symbol Digit Total correct in 90 seconds (NC01a) |  | 1349 | neurocog |
| NEUROCOG | DSYMTSCO | Symbol Digit T-score, model based T-score adjusted for baseline, second, third, later administration, age, education, race, WRAT (Based on MACS) |  | 1308 | neurocog |
| NEUROCOG | DSYM\_REC | Symbol Digit Delayed Recall Score (NC01a) |  | 1336 | neurocog |
| NEUROCOG | TRLA | Trails A--time to complete in seconds (NC01a) |  | 1373 | neurocog |
| NEUROCOG | TRLATSCO | Trails A T-score, model based T-score adjusted for baseline, second, third, later administration, age, education, race, WRAT (Based on MACS) |  | 1327 | neurocog |
| NEUROCOG | TRLB | Trails B--time to complete in seconds (NC01a) |  | 1326 | neurocog |
| NEUROCOG | TRLBTSCO | Log10 Trails B T-score, model based T-score adjusted for baseline, second, third, later administration, age, education, race, WRAT (Based on MACS) |  | 1285 | neurocog |
| NEUROCOG | TSCORE\_E | Executive Domain (trlbtscore strp3timtscore) T-score, model based T-score adjusted for baseline, second, third, later administration, age, education, |  | 1285 | neurocog |
| NEUROCOG | TSCORE\_S | Speed Domain (dsymtscore strp2timtscore) T-score, model based T-score adjusted for baseline, second, third, later administration, age, education, rac |  | 1308 | neurocog |
| NEUROCOG | WRAT\_SS | Age-adjusted WRAT Standard Score. (NC02A) |  | 1334 | neurocog |
| NEUROCOG | YRSEDUCN | Years of Education (NC03) |  | 1381 | neurocog |
| RISKSUM | APRI | Liver fibrosis scores, Aspartate aminotransferase/platelet ratio index(APRI), AST/AST\_ULN)\*(100/platelet) | 2101 | 1438 | risk factor |
| RISKSUM | BF | body fat calculated as weight\_kg-FFM | 1709 | 24 | risk factor |
| RISKSUM | BMI | Body Mass Index (kg/meter\*\*2) | 2132 | 2036 | risk factor |
| RISKSUM | DIASTOLI | Diastolic blood pressure (mmHg) | 2129 | 2049 | risk factor |
| RISKSUM | EGFR1 | Estimated Glomular Filtration Rate, ml/min/1.73 m^2 {Modification of Diet in Renal Disease [MDRD] Study equation: GFR = 186 x (Pcr)^(-1.154) x (age)^ | 2167 | 2075 | risk factor |
| RISKSUM | EGFR2 | Estimated Glomular Filtration Rate, ml/min/1.73 m^2 {CKD-EPI equation, Annals of Internal Medicine 2009: GFR = 141 x min(Scr/k,1)^a x max(Scr/k,1)^-1 | 2167 | 2075 | risk factor |
| RISKSUM | FFM | fat free mass calculated as 0.8787\*((height\_cm\*\*1.9748)/( (impedance\*\*0.4851)\*22.22))+ (0.0813\*weight\_kg)+0.0709 refered in Dr. Kotler'Prediction of bo | 1709 | 24 | risk factor |
| RISKSUM | FIB4 | FIB4-4, a non-invasive index of hepatic fibrosis: (age\*AST)/(platelet\*sqrt(ALT)) | 2101 | 1437 | risk factor |
| RISKSUM | GLUCOSE | Fasting glucose | 1501 | 1520 | risk factor |
| RISKSUM | HEIGHT | Height in meters | 2287 | 2225 | risk factor |
| RISKSUM | HEMOGLOB | Hemoglobin A1c | 1823 | 1891 | risk factor |
| RISKSUM | HMEAS | Hip circumference (cm) | 1841 | 1809 | risk factor |
| RISKSUM | HOMAIR | Insulin resistance using the Homeostasis Model Assessment (HOMA) method: (insulin x glucose)/405 where insulin is measured in uIU/mL and glucose in m | 1423 | 1448 | risk factor |
| RISKSUM | PBF\_BIA | Percentage body fat on BIA(Bioelectrical Impedance Analysis) calculated as (BF/weight\_kg)\*100 | 1709 | 24 | risk factor |
| RISKSUM | SYSTOLIC | Systolic blood pressure (mmHg) | 2129 | 2049 | risk factor |
| RISKSUM | WEIGHT | Weight in kilograms (kg) | 2132 | 2036 | risk factor |
| RISKSUM | WMEAS | Waist circumference (cm) | 1841 | 1811 | risk factor |
| SOCDEM | AGEATBL | Age at Baseline visit | 2175 | 2083 | Sociodemographics |
| SOCDEM | CESD | Overall depression score (F26/C1-C20) | 2128 | 2032 | Sociodemographics |
| SOCDEM | COGNF | Cognitive function (F26/B8b-c) | 2142 | 0 | Sociodemographics |
| SOCDEM | EMOTL | Emotional wellbeing (F26/B8d-e,h) | 2142 | 0 | Sociodemographics |
| SOCDEM | HLTHP | Health perception (F26/B1,B9a-b) | 2146 | 0 | Sociodemographics |
| SOCDEM | HLTHR | Health rating (F26/B10) | 2139 | 0 | Sociodemographics |
| SOCDEM | NDRNKWK | Number of drinks/week since last visit | 2166 | 2065 | Sociodemographics |
| SOCDEM | PAIN | Pain (F26/B3,B6) | 2145 | 0 | Sociodemographics |
| SOCDEM | PHYSF | Physical function (F26/B7a-d) | 2144 | 0 | Sociodemographics |
| SOCDEM | QLINDX | Quality of life health index scale (.2\*physf +.17\*pain +.28\*enfat +.2\*emotl + .05\*soclf + .1\*rolef) | 2141 | 0 | Sociodemographics |
| SOCDEM | ROLEF | Role function (F26/B2,B5) | 2146 | 0 | Sociodemographics |
| SOCDEM | SMKGRP | History of Smoking cigarettes status | 2173 | 2083 | Sociodemographics |
| SOCDEM | SMKHIST | Number of years smoked cigarettes | 2173 | 2083 | Sociodemographics |
| SOCDEM | SOCLF | Social function (F26/B4,B8a) | 2145 | 0 | Sociodemographics |
| XXXXX | gal3bp | inflammation marker |  |  |  |
| XXXXX | gal3 | inflammation marker |  |  |  |
| XXXXX | scd14 | inflammation marker |  |  |  |
| XXXXX | scd163 | inflammation marker |  |  |  |
| XXXXX | il6 | inflammation marker |  |  |  |
| XXXXX | cd4\_cd57p\_cd28n | inflammation marker |  |  |  |
| XXXXX | cd4\_hladrp\_cd38p | inflammation marker |  |  |  |
| XXXXX | cd8\_cd57pn\_cd28n | inflammation marker |  |  |  |
| XXXXX | cd8\_hladrp\_cd38p | inflammation marker |  |  |  |
| XXXXX | CRP\_W05015 | inflammation marker |  |  |  |

1. **Back up**

\_\_2\_\_

Table 1. basic characteristics

\_\_1\_\_

background:

Antiretroviral threatments (ART) use의 사용은 people living with HIV (PLWH) 에게서 HIV-infection을 chronic disease 와 같이 만들어 정상인과 같은 삶을 살수 있다록 도와주고 있다. {Deeks, 2013 #365} 하지만 ART의 사용자의 경우 (나이와 AIDS에 상관없이? belong to the long term ART use occur longer ART toxicity) 정상인에 비하여 더 chronic complication and comoridities의 risk 가 높다.{} {Negin, 2012 #362}

또한 ART 의 사용은 PLWH의 몸안의 metabolism 을 dramatic하게 변화 시킨다. {공씨 논문, ART and lipids change}

이러한 ART 사용은 metabolic complications를 야기 한다. {Nakaranurack, 2018 #364} 이러한 논문들은 일반적인 factor (e.g., age, sex, CD4 level)등을 보았지 metabolomics 수준에서 본 데이터들이 아니다.

또한 우리의 기존 연구에 따르면 HIV 감염과 다양한 질병관련 혹은 면역 관련 factor들과 영향이 많이 있음을 알 수 있다. 예를 들어 {로버트, 데이비드} 같은 연구들이 특정 factor들이 disease risk increase와 관련이 깊음을 확인 할 수 있었다.

**previous evidence**

based on their previous studies, we focused on the disease related risk factor and continuous variables. {} most important variables (e.g., BP, lipids, etc.) role key risk factor in the several diseases. {}

these prior studies provide strong evidence of a role for HIV status and related metabolites in the development of comorbidities, but further research is warranted. altered metabolism is observed in HIV-infected individuals, potentially triggering chronic inflammation, T cell activation, and disrupted metabolite catabolism, and thus finally leading to high comorbidities risk.

Life expectancy in people living with HIV (PLWH) has increased in the past decades, since the introduction of highly active antiretroviral treatment (ART). {Peterson, 2019 #361}

according to Negin et al study, aging is important factor in comorbidities.{Negin, 2012 #362} Also ART use might be associated with increased metabolic risk factor.{valcour et al}

Aim:

methods:

results:

The prevalence of comorbidities is substantially high in the HIV-infected individuals than non-infected group.

conclusion:

the question of multimorbidity is critical to the future care and treatment model of HIV.?

chronic comorbidities, which may be due to chronic inflammation from long-term HIV infection as well as ART toxicities exacerbated by aging.{Negin, 2012 #362}

the finding of this study may help us to better understand association of metabolomic signatures with disease related factors in PLWH and find way to lower their risk of chronic HIV-related comorbidities.

? metabolic comorbidities

through these finding, identified metabolomic signature in HIV-infected individual is discovered the relationship disease related factor and metabolites. Defined relationship with significant metabolites and comorbidities related factors.

the finding of this study may help us to better understand association of metabolomic signatures with disease related factors in PLWH and find way to lower their risk of chronic HIV-related comorbidities.

\*Our main hypothesis is that alteration in GMB during HIV-infection lead to inflammation/immune activation and adverse metabolomic profiles. identifying the metabolic and immune cell transcriptome features that underlie this phenomenon is our underlying goal.\*

\*The prevalence of comorbidities is substantially high in HIV infected individuals, who receiving ART use. Clinical monitoring and effective management of these comorbidities and metabolic complications are recommended, especially in HIV-infected patients who present with these associated metabolomic signature.\*