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2019

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UNIVERSITY OF CALIFORNIA

Los Angeles

The Effect of Erectile Dysfunction Drugs Use on the Immune System in the

Multicenter AIDS Cohort Study (MACS)

A dissertation submitted in partial satisfaction of the

requirements for the degree of Doctor of Philosophy

in Epidemiology

by

Jee Won Park

2019

ABSTRACT OF THE DISSERTATION

The Effect of Erectile Dysfunction Drugs on the Immune System in the Multicenter AIDS

Cohort Study

by

Jee Won Park

Doctor of Philosophy in Epidemiology

University of California, Los Angeles 2019

Professor Roger Detels, Chair

The relationship between erectile dysfunction drugs and the immune system is unclear. There

have been animal studies in the past to support a biological rationale for these drugs to have

immunomodulatory effects on the levels of immune markers. This study examines the effect

of erectile dysfunction drugs in both HIV positive and negative men who have sex with men.

Over one thousand men with semi-annual visits from the Multicenter AIDS Cohort Study

provided data ranging from demographics to sensitive information on sexual activities,

CD4/8 T-cells and inflammatory biomarkers. Study participants with age ranging from 19 to

70 were only included from 1998 onward, which was when the erectile dysfunction drugs

were available. Bivariate random-intercept models were used to assess correlations between

erectile dysfunction drug use and other behavioral factors over time at both population and individual levels. Recreational drugs use and engagement in risky sexual activities, such as unprotected anal intercourse, were positively associated with erectile dysfunction drug use. By applying marginal structural models for causal inference in a complex longitudinal setting, we provided evidence to show improved immune capacity, exhibited by increased CD4 cell counts and percentages, in HIV positive men who reported both short and long-term drug use. We showed further evidence of anti-inflammatory effects of using erectile dysfunction drugs and reduced levels of pro-inflammatory markers in both HIV positive and negative men. We conclude that erectile dysfunction drug use demonstrated favorable immunomodulatory effects in men with and without HIV infection.

The dissertation of Jee Won Park is approved.

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LIST OF ACRONYMS

BMI: Body Mass Index

CDC: Center for Disease Control and Prevention

CI: Confidence Interval

ED: Erectile dysfunction

GHB: gamma-Hydroxybutyric acid

HAART: Highly Active Anti-Retroviral Therapy

HIV: Human Immunodeficiency Virus

MACS: Multicenter AIDS Cohort Study

MD: Mean Difference

MSM: Men who have sex with men

N: Number

OR: Odds Ratio

PCP: Phencyclidine

R: Correlation coefficient

SD: Standard Deviation

SE: Standard Error

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere gratitude to my advisor and committee chair, Dr. Roger Detels, for his tremendous support during my Ph.D. study. His patience, knowledge, wisdom, and good humor has guided me to research and complete this dissertation. I have developed into a much better researcher and person through his care and mentorship.

I would also like to express gratitude to the rest of my committee members, Dr. Onyebuchi Arah, Dr. Otoniel Martinez-Maza and Dr. Robert Weiss, for their insightful feedbacks, continuous support and encouragement. Besides my dissertation committee members, I am sincerely grateful to Dr. Roberta Malmgren, Dr. Sung-Jae Lee, Dr. Katherine Hoggatt, Dr. Roch Nianogo and Dr. Josh Quint for their encouragement and valuable interactions during my research. A special acknowledgement must be made to Wendy Aft and Joy Miller who works so hard for the students in the Department of Epidemiology.

I would like to thank my friends and colleagues, Vahe Khachadourian, Amy Chai, Adva Gadoth, Shelley Jung, Hayley Ashbaugh, and Mahmoud Gaddoury, for the productive days and entertaining evenings spent together during our study at Fielding School of Public Health. A special thanks goes to my good friend, Ricky Rivers; his presence alone was immensely supportive.

Last but not least, I would like to thank my parents, Sam Chul Park and Soon Ok Kim, my sister, Monica, and especially Hyorim Lee, whose motivation and support kept me on track throughout this journey.

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CHAPTER 1

Introduction

1. Introduction

1.1 Epidemiology of HIV

Since the beginning of the epidemic in 1983, HIV was and remains one of the most significant public health issues in the world. By 1995, AIDS-related mortality was the leading cause of death among adults aged 25 to 44 years old. However, the availability of multidrug therapy for HIV led to a decline in the number of deaths, and according to the CDC in the United States (US) in 2015, AIDS-related illnesses were the 9th leading cause of death for adults between the ages of 25-44 (*CDC, 2017*).

Global HIV statistics in 2017 showed an estimated 36.7 million people were living with HIV, with 1.8 million people being newly infected. Although HIV incidence in children declined by 47% since 2010, among adults, new infections declined approximately 11% (*UNAIDS, 2017*). Eastern and southern Africa accounted for 43% of all new HIV infections, but other regions such as Asia and the Pacific were also significantly affected by HIV/AIDS. AIDS-related deaths were estimated to be around 1.0 million in 2016, and in total, 35 million people have died since the start of the epidemic. This does not only reflect health burdens, but also economic burdens. In response to the AIDS epidemic in low- and middle-income countries, an estimated US$ 19.1 billion was invested in 2015, and the UNAIDS predicted US$ 26.2 billion and US$ 23.9 billion will be required for 2020 and 2030, respectively.

In the United States, there was an estimated 1.1 million persons over the age of 13 living with HIV infection at the end of 2015, including 162,500 (15%) persons with undiagnosed infection (CDC, 2018). In 2014, approximately 6,721 people died from HIV/AIDS, and as mentioned previously, HIV continues to remain within the top ten leading cause of deaths and especially affected certain sub-populations in the United States. According to the CDC Statistics in 2016, African Americans accounted for 44% of the new

HIV diagnoses while they only made up 12% of the population. By transmission category, male-to-male sexual contact accounted for 67% of new HIV diagnoses.

1.2 Epidemiology of HIV among MSM population

MSM are at a high risk of HIV mostly due to their sexual risk behaviors. Most gay and bisexual men acquire HIV infections through anal receptive sex, which has the highest risk for HIV transmission. In many high-income countries, the overall HIV epidemic trends are declining except in MSM population. In the United States, MSM remain the group most heavily affected by HIV, with an estimated rate increasing at around 8% per year since 2001. MSM made up only 2% of the U.S. population in 2014, but accounted for approximately 70% of new infections. In 2016, CDC reported an estimated 26,570 new infections among MSM and in the same report, it was noted that this accounted for 83% of the diagnoses in the male population. Furthermore, Black MSM accounted for the largest number of new infections, with 10,223, followed by Latino and White MSM (CDC, 2017). In a study that pooled data from peer-reviewed publications on the burden of HIV in MSM, HIV prevalence among MSM population was found to be within a range of 3.0% in the Middle East and North Africa region, to 25.4% in the Caribbean. In North America, it was approximately 15.4% (Beyrer et al, 2012; Figure 4). In the same study, incidence for HIV among MSM showed increasing rates in countries such as China and Thailand, while other countries that had data on incidence showed no decline, which suggested that these levels of incidence were sufficient for HIV epidemics to continue in the MSM population. Since the start of the epidemic, approximately 311,087 MSM with an AIDS diagnosis have died, including an estimated 5,380 in 2012 (CDC, 2015).

1.3 Erectile Dysfunction (ED) and ED drugs

There are currently three drugs approved by the FDA for erectile dysfunction.

Sildenafil (Viagra) was approved by the FDA and became available on the market in 1998 as an erectile dysfunction (ED) drug, then followed Vardenafil (Levitra) and Tadalafil (Cialis) in 2003. These drugs were first tested for the treatment of pulmonary arterial hypertension, however, as a selective and potent inhibitor of phosphodiesterase type 5 (PDE-5), which increases cyclic guanosine monophosphate (cGMP) levels, they were found to be more effective in the treatment of ED (Barnett, 2006).

Erectile dysfunction is common among men with HIV (Romero-Velez, 2014). Prevalence of ED varies from 30-60% and has been reported to be frequent even in men aged less than 40 years (Santi, 2014). Worldwide, ED affects over 180 million men (Jackson, 2005). Among the MSM, prevalence of ED ranged from 33 to 74% among HIV positives compared to 0 to 18% in the general population sample. Within the Multicenter AIDS Cohort Study, prevalence of ED was found to be around 18%, with higher prevalence among the HIV positive compared to HIV negative men in the sample (Hart et al, 2012). Although the exact numbers of men who use the drugs are unknown, Pfizer has estimated that over 25 million men globally have been prescribed sildenafil alone. In addition, these ED drugs are not only used by men from prescriptions for the treatment of ED, but also as a sexual enhancement aid among men without medical indication (Harte and Meston, 2011). In a community-based anonymous survey among MSM in San Francisco, 32% had ever used Viagra (Chu et al, 2003), and other studies among men seeking public STD services in San Francisco and college men in the United States, found MSM were approximately 3 to 4 times as likely to report recreational ED drug use, compared to heterosexual men (Kim et al, 2002; Harte and Meston, 2011). In addition, as the population ages, the uses of ED drugs are expected to increase.

There were several studies that have looked into the effects of these drugs on the

immune function in both animal models and humans, but there were no studies that have examined the long-terms effects. PDE-5 inhibitors are modulators of anti-tumor immune response, and have been shown to induce apoptosis in different human tumors (Serafini, 2006). Studies by Califano et al. (2015) and Weed et al. (2014) have shown that tadalafil enhances systemic immunity by increasing T-cell proliferation, both CD4 and CD8 T-cells, as well as inhibiting immunosuppressive function by suppressing inducible NOS and arginase-1 (inhibits immunosuppressive function on MDSC's). Other effects of PDE-5 inhibitors included an increase in tumor capillary permeability, which leads to anti-tumor efficacy (Black, 2008).

Main adverse effects of ED drug usage include headaches, flushing, dyspepsia, nasal congestion (Barnett, 2006). In addition, more serious adverse effects have been reported, such as vision loss, hearing loss (McGwin, 2004) and cardiovascular effects (Safarinejad, 2004).

1.4 Immune Markers

HIV/AIDS is characterized by several immune responses to an infection with the virus. During an infection, the innate (Natural Killer cells, Antigen Presenting Cells, etc.) and adaptive (CD4 helper T lymphocytes and CD8 cytotoxic T lymphocytes) immune systems are activated. Furthermore, the general immune activation by cytokines reacts to the viral replication inside the host. So, HIV is associated with an increased expression of pro-inflammatory cytokines, such as TNF-alpha and IL-6, which are then associated with an up-regulation of HIV replication. In addition, immune-regulatory cytokines such as IL-2 and IL-12 are disrupted and lost, which then are not able to modulate an effective cell-mediated immune response via the T cells (Reuter et al, 2012). Hence, circulating biomarkers of immune activation and inflammation can be measured and used in studies to examine the immune function and its responses.

Common biomarkers used in epidemiological studies include TNF-alpha, IL-6 and C-reactive protein (CRP). These have been studied intensively in the past and there is a somewhat sufficient knowledge on the mechanisms of the biomarkers. However, new biomarkers are constantly being discovered and studied, and add to the current knowledge of a complex immune response system. Moreover, with the development of multiplex assays, it is now possible to examine multiple biomarkers with a small serum sample. There are several different classification methods that can be used, but broadly, the biomarkers of the immune function can be classified into three categories: 1) cytokines, 2) chemokines, and 3) CD antigens/soluble receptors.

Cytokines are soluble factors, which are produced by immune or non-immune cells. There are many different types of cytokines, which are produced by many different cells. In response to an innate and specific immune response, cytokines are released by cells to mediate and regulate a response. For example, macrophages can secrete cytokines such as IL-1, TNF-alpha, IL-6, IL-8 and IL-12 once they are activated. The released cytokines have both local and systemic effects on nearby and surrounding cells. IL-1 induces local inflammation, aids in the production of neutrophils and induces a fever, whereas IL-6 also induces inflammation and it regulates B and T cell functions. However, there are also cytokines that are present in very low or undetectable levels in circulation. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is produced by various cell types such as macrophages, mast cells, T cells, fibroblasts and endothelial cells. GM-CSF is secreted mainly in response to immune activation and other cytokines that mediate inflammation, such as the IL-1 and IL-6. Under normal conditions, GM-CSF in circulation is at a low level, and significant increases in the level of the biomarker can be seen in certain circumstances of immune activation.

Chemokines are a type of cytokines but produced as a chemo-attractant cytokine. This means that cells are attracted to the chemokines, which are located at the sites of infection or inflammation (Pease and Williams, 2009). These biomarkers include all the CC and CXC receptors, which play an important role in immune responses.

Finally, CD antigens or soluble receptors are cytokine receptors that exist in soluble form in serum or plasma. These soluble receptors are a truncated form of the membrane receptors with the ligand-binding extracellular portion intact. Since these receptors compete with the membrane receptors for cytokines, they inhibit the binding and activity of their respective cytokines (Fernandez-Botran, 1991). For example, CD 126, also known as sIL-6R is a receptor for IL-6, which in soluble form can bind to gp130 and promote IL-6 induced response.

1.5 Aims and hypotheses

This study focused on the question of whether there were any effects of ED drug use on the immune capacity and functions in homosexual men. The effect of ED drug on the immune responses have been already mentioned, however, this study aimed to find which biomarkers were affected by ED drugs and how the level of these markers changed over time. The hypothesis for the research question was that subjects who have reported use of ED drugs have improved immune capacity, represented by the levels of CD4 and CD8 T lymphocytes, and immune function, represented by the levels of biomarkers. In particular, inflammation-associated biomarkers (pro-inflammatory markers) would be decreased in those who were ED drug users.

For the purpose of this research, three specific questions among the MSM population were examined:

1. Who were the people using ED drugs, i.e. characteristics of subjects with respect to ED drug use and identification of important variables that were correlated with ED drugs?

2. What were the overall effects of ED drugs on the immune capacity (immune cells)?

3. What were the overall effects of ED drugs on the immune function (inflammatory biomarkers)?

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CHAPTER 2

Methods

2. Methods

2.1 Study Population

2.1.1 MACS (The Multicenter AIDS Cohort Study)

The Multicenter AIDS Cohort Study (MACS) is an ongoing prospective study of HIV-1 infection among men who have sex with men (MSM) in the United States (Kaslow et al, 1987; Detels et al, 1992). A total of 4,954 men were recruited in 1984 in four metropolitan locations in the United States: Baltimore MD /Washington D.C., Chicago IL, Los Angeles CA, and Pittsburgh PA. Since then, additional recruitment was made in waves during 1987-90, 2001-03 and 2010 with 668, 1,350 and 115 subjects, respectively. Therefore, since the start of the study in 1984, there was a total of 7,087 MSM enrolled. Eligible persons had to be sexually active, 18 years or older, and free of an acquired immunodeficiency syndrome (AIDS)-defining illness. The purpose of the MACS was to examine the natural history of HIV infection and to identify the risk factors (Detels et al, 1992).

During semi-annual study visits, subjects were evaluated by standardized questionnaires, some administered by the use of ACASI, to collect information on demographics, medical history, health services, behaviors and medications. In addition, physical examinations were conducted, as well as tests for psychosocial factors, and neuropsychological screenings were performed. Laboratory testing and storage was also part of study, which includes the collection of blood for measurements of immune cells and biomarkers (McKay et al, 2016; *MACS dossier*). Institutional review boards at each study center approved the MACS study protocols and informed consent was obtained from all participants.

2.1.2 ARRA1

The purpose of the ARRA1 study was to examine the level of biomarkers among all

HIV seroconverters and HAART initiators in the MACS during the course of the infection. The ARRA1 study has approximately 14,000 serum specimens that were tested from the MACS and includes four groups: 1) Seroprevalent HAART initiators (n=939), 2) Seroconverters (n=542), 3) Long-term non-progressors (n=57), and 4) HIV negative controls (n=250). Serum samples from MACS person-visits were chosen for inclusion in the study if the men had known HIV seroconversion dates and had annual lab visits from immediately before and after HAART initiation for all HAART users. Then, pre-HAART visits from HIV seroprevalent men with labs within two years before HAART initiation were sampled. In addition, follow up samples for post-HAART person-visits were taken biannually. Controls were also selected from HIV-uninfected men who were of similar age and race from 1984-2009.

For the purpose of the study, the study cohort was restricted to those who were in the study since the ED drugs were first available, which was from the year 1998 (visit 28) and onwards. This included 1,636 men from the MACS data between visit number 28 to 64 (1998-2016) with a total of 44,712 observations. From the 1,636 subjects, 1,582 were also in the ARRA-1 study with 15,328 observations from visit number 28 to 52 (1998-2010). Therefore, in the analyses of immune cells (CD4 and CD8), subjects from the MACS were used, while the ARRA-1 subjects and observations were used for immune markers.

2.2 ED drug variable

The variable for ED drug use comes from the MACS. During the semiannual visits, MACS participants completed a questionnaire, which included sections on ED and ED drugs. Exposure variables could be defined in several ways using the diagnosis with erectile dysfunction and usage of erectile dysfunction drugs questions. For the present study, ED drug use was defined as those who answered "Yes" using the self-reported drug variable in the

MACS questionnaire. The self-reported responses were collected from questions on "other medications" as well as ED drug prescribed specific for ED diagnosis. In addition, ED drugs that were not prescribed for ED was also collected (Ostrow et al, 2011). ED drugs included Viagra (sildenafil), Cialis (tadalafil) and Levitra (vardenafil). It was not possible to distinguish between the different ED drugs as there was no question asking which drug the participants used.

2.3. Outcome variables

Two multiplex assay platforms were used to quantify the serum biomarkers of immune activation and inflammation in the ARRA1 study. For the measurements of nine cytokine concentrations: IL-1b, IL-2, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, GM-CSF and TNF-α, the Meso Scale Discovery (MSD, Gaithersburg, MD) system using the Ultra-sensitive Human Pro-inflammatory 9-Plex Kit was used. For the measures of seven chemokines: CCL2, CCL4, CCL11, CCL13, CCL17, CXCL10, and IL-8 (CXCL8), MSD Ultrasensitive Human Chemokine 7-Plex Kit was used according to the manufacturer's protocols. The MSD is an electrochemiluminescence-based 96-well format solid-phase assay (Wada et al, 2015; McKay et al, 2017). IL-8 (or CXCL8) is categorized as both cytokine and chemokine; hence, it was measured in both kits. Both cytokines and chemokines were measured at the same study site in Baltimore, MD.

The soluble receptors were measured at a different study site in Los Angeles, CA. The Luminex platform (Luminex, Austin, TX) was used according to the manufacturer's protocol (R&D Systems, Minneapolis, MN) to measure the six soluble receptors: (s)CD14, sCD27, sgp130, sIL-2Rα, sIL6-R, sTNF-R2, as well as one cytokine, B-cell activating factor (BAFF), and one chemokine, CXCL13, using a single lot of assay kits, to eliminate lot-to-lot variability. The Luminex platform is a fluorescent bead-based assay and the data were

collected and analyzed using BioPlex 200 apparatus and BioPlex Manager software (Bio-Rad, Hercules, CA). With both platforms, all samples from an individual were tested on one plate to minimize variability. Each plate contained samples from both HIV-infected and HIV-uninfected men.

Many cytokines, such as the before-mentioned GM-CSF, are difficult to measure because they are only present in very low concentrations in circulation, whereas serum chemokine and soluble receptor concentrations are found at a higher and detectable level. Hence, for the purpose of the study, biomarkers will be assessed on the level of detection, and those with low level of detection may be excluded from the analyses.

Therefore, the outcome variable can be classified broadly into two categories with further sub-categories within.

1. Immune capacity: (outcome of T-cells)

     i. CD4 and CD8 count

     ii. CD 4 percentage

     iii. CD4:CD8 ratio

There are emerging evidences regarding the clinical relevance of CD4:CD8 ratio independent of absolute values of either cell type (Serrano-Villar et al, 2013; Lu et al, 2015; McBride and Striker, 2017). Also, percentage of CD4 T cells has been known to be an independent marker of immune function (Taylor et al, 1989).

2. Immune function:

     i. Cytokines (10): BAFF, IL-1b, IL-2, IL-6, IL-8pro, IL-10, IL-12p70, IFN-γ, TNF-α, GMCSF

     ii. Chemokines (8): Eotaxin/CCL11, MCP-1/CCL3, MCP-4/CCL13, MIP-1b/CCL4, TARC/CCL17, BLC/BCA1/CXCL13, IL-8/CXCL8, IP-

10/CXCL10

iii. Soluble receptors (6): sIL-2Rα, sIL-6R, sTNF-R2, sCD14, sCD27, sGP130/CD130

Assessed over time, with repeated measures, the changes in the level of biomarkers listed above can act as proxy measures for the immune function.

2.4. Statistical Analysis

The MACS data is a complex longitudinal data set that includes time-varying exposure, confounders and outcome. Observations in individuals are correlated and not independent. To assess whether one variable was associated, or correlated, with the outcome variable, the usual regression methods, such as logistic regression, would be used. However, this would be inappropriate because it would ignore the correlations between observations and also the covariance structure of the data. On the other hand, if the correlations were correctly taken into consideration, the variability of the outcome between individuals and within individuals can be assessed (Das et al., 2004). Models such as the generalized linear mixed model (GLMM) can use these correlated data with multiple binary outcomes, so this model was selected as the main method for analysis in the present study. A univariate random intercept model, with one outcome and other variables as predictors, will show whether the predictors are associated with the outcome. However, a bivariate random intercept model (Weiss, 2005) using the GLIMMIX procedure in SAS can be utilized to assess whether a response variable was correlated over time with ED drug use. This model showed whether the two outcome variables were correlated with each other on a population level over time. The model chosen has an advantage that it can show whether two variables are correlated on an overall level, as well as whether the variables of interest move up and down together within individuals. It can do this by jointly modeling the two variables as a bivariate response

that was repeatedly measured over time. In addition, variables with different number of observations can still be used in the model without losing information.

Response variables that were tested for correlation with ED drugs were the substance use, sexual behaviors, and medication use variables, and these models were run separately for each response variable. Fixed-effect covariates were selected, a priori, into the model as predictors to adjust for important confounding variables, and age, which was re-centered using the median, and re-scaled by 10 years, was used as the time variable. Using the variance and covariance obtained from the results of the bivariate random intercept model, a correlation coefficient, ρ, was calculated by the following formula:

$$\rho = \frac{\text{cov}(x,\ y)}{\sigma_x\ \sigma_y}$$

where x and y represents the two outcome variables, and σ is the variance. Here, the correlation refers to the population-level average. A correlation coefficient in the range of 0-0.3 was considered a weak correlation, moderate if it was between 0.3 to 0.5, and greater than 0.5 was a strong correlation.

To examine whether the correlation between ED drug use and other variables were present within individuals, GLMM of ED drug with confounding variables was performed to obtain the residuals. Then, these residuals were included as a predictor in the bivariate random-intercept model and the resulting estimate for the residual variable was used as an indication of the within-subject association. So, a positive coefficient of the residual would suggest a positive association between the ED drug use residual and the response variable, i.e. within subjects, increase in response variable use also results in higher ED drug use over time. In summary, the steps required for the within individual analysis were as follows:

1. Calculate the predicted mean of ED drug use

2. Calculate the residual (difference of predicted and observed mean of ED drug use)

3. Regress ED drug use with the other response variables by including the residual obtained from Step 2 as a predictor

4. Read off the estimate of the residual variable

5. Repeat steps 1-4 for other response variables

Steps 1-2 can be done in one step in SAS by adding the PRED=, or PREDICTED=, and RESID=, or RESIDUAL=, option in the OUTPUT statement to the GLIMMIX procedure. This procedure was repeated by dividing the data into HIV positive and negative to examine whether the correlations differed among the two groups. To fit the bivariate random-intercept model, a single response variable was created to represent both outcome variables. Furthermore, to help with model convergence issues, MAXITER= and TECH=newrap was included in the NLOPTIONS option of some models. MAXITER increased the number of iterations for the model to converge and TECH specified the Newton-Raphson optimization technique to calculate the maximum-likelihood estimators. Alpha level of 0.05 was used for all analyses.

To estimate the causal effect of ED drugs on CD4 and CD8 cell counts, we implemented g-computation (*Robins, 1986; Robins, 2000*) in a longitudinal setting shown in the directed acyclic graph (DAG) in Figure 1. Time-varying covariates in such longitudinal settings are confounding variables that must be adjusted for later exposures, (A_t). But these variables are also on the indirect path from previous exposure (A_{t-1}) to the later outcome (Y_t), in which case they should not be adjusted for in the analysis. This is especially the case when there is confounding by indication (*Hernan et al., 2000; Robins et al., 2000; Hernan et al., 2001*), which may be the case in this study with the use of ED drugs. By using a marginal structural model, it was possible to eliminate such time-varying confounding effects while

preserving the intermediate paths. Figure 2 shows the intervention DAG after the simulation. Here, confounding effects by the time-dependent variables are eliminated while the intermediate pathways from previous exposure to the next outcome remain.

If we let A_t be exposure, Y_t be outcome, L_t is a set of covariates (confounding variables) where t represents time/visit (t=1, 2,…, 21), Y_a is the potential outcome of Y when A is set to a and *newA* is when we set A to be a. Firstly, we specified and fitted the models for the outcome of interest, $E(Y_t|A_t, L_t, Y_{t-1})$, and the models for time-varying confounders, L's: $P(L_t| A_{t-1}, L_{t-1})$, using the observed Y's, A's, and L's in PROC GENMOD and saved the regression coefficients. Next, we created two copies of the data, where one was given *newA*=1 (all subjects took ED drugs) and the other was given *newA*=0 (all subjects did not take ED drugs). Then, using Markov Chain Monte Carlo simulation with 1,000 repetitions, we created *newL*'s and new potential outcomes, $E(Y_a|newA, newL)$, in each time point using the saved parameters. Finally, we regressed Y_a on *newA* to estimate the causal mean difference using PROC GENMOD. Confidence intervals were computed by summarizing the results over the repetitions using the 2.5[th] and 97.5[th] percentile as the lower and upper confidence limits. This study included multiple A's, L's and Y's over time, so construction of new variables was repeated for each time point to account for these extra terms. Interactions between ED drug use and time-fixed variables, such as age, race and education were included in the model. Interactions terms between ED drugs and other confounding variables were tested but showed no statistical significance and were not included. Data were analyzed in SAS 9.4 (SAS Institute Inc., Cary, North Carolina).

CHAPTER 3

Manuscript #1

Characteristics and patterns of erectile dysfunction drug use among men who have sex with men: Multicenter AIDS Cohort Study (MACS)

Abstract

Background: Erectile dysfunctions (ED) drugs are associated with other risky behaviors such as substance use and other sexual activities, and they have been well documented in the past. However, the patterns of long-term erectile dysfunction drug use among men who have sex with men are limited

Methods: Subjects who were in the Multicenter AIDS Cohort Study (MACS) during 1998 to 2016 were included in the study (n=1,636). We used a bivariate random-intercept model to model ED drug use with other behavioral factors to assess the relationships between the two outcomes over time on a population level. We further examined the associations between ED drug use and other factors at an individual level.

Results: Average ED drug use was weakly correlated with marijuana use (r=0.19), poppers (r=0.27), and stimulants (r=0.25) in HIV positive men. Testosterone use (r=0.32), risky sex (r=0.40), PIAI (r=0.40) and UIAI (r=0.43) all showed moderate correlations over time. HIV negative subjects showed similar correlations, as the average marijuana use (r=0.19), stimulants use (r=0.22) were weakly correlated with ED drugs but were moderately related with risky sex (r=0.36), PIAI (r=0.22), and UIAI (r=0.18) over time. Significant within-individual associations were observed in both groups of men between ED drug use and risky sexual activities.

Conclusion: Men in the MACS study who reported the use of ED drugs were also, on average, likely to use recreational drugs and engage in high-risk sexual activities. Within-individuals, average ED drug use was also positively associated with sexual activities.

3.1 Background

Erectile dysfunction (ED) is common in HIV (Romero-Velez et al., 2014) and independently associated with HIV in men who have sex with men (MSM) (Dijkstra et al., 2018). Since its approval by the Food and Drug Administration (FDA) in 1998, the use of ED drugs has been documented in the past to be more common among men who have sex with men (MSM) than heterosexual men (Kim et al, 2002).

ED drugs are also used by younger men as a recreational drug, mixing ED drugs with other substances, such as ecstasy (Fisher et al, 2006). Use of recreational drugs, including ED drugs, has been reported at high rates among different subpopulations of MSM regardless of age and socioeconomic status (Mansergh et al, 2004; Colfax et al, 2004; Purcell et al, 2005). Moreover, concurrent use of these drugs was frequently reported (Mansergh et al, 2004). There is evidence of widespread recreational drug use among MSM including marijuana, cocaine, ecstasy, and Viagra (Purcell et al, 2005; Halkitis et al, 2005; Ruf et al, 2006). The majority of MSM from an Australian community cohort study reported that use of recreational drugs was part of their lifestyle (Ruf et al, 2006). In another cross-sectional study of young heterosexual, bisexual, and gay men in the U.S., 5% of the participants reported using ED drugs mostly for recreational purposes (Harte and Meston, 2011). In the same study, more than half of the men reported using ED drugs together with marijuana, and some with ecstasy, methamphetamines, and cocaine.

ED drug use is closely related to increase in risky sexual activities. Association between ED drugs and sexual behaviors were commonly reported in studies that examined recreational drug uses. In a community-based sample of MSM, there was a strong association of ED drugs with the use of illicit drugs, and risky sexual behavior (Chu et al, 2003). Insertive anal intercourse was a major predictor of the use of ED drugs, knowledge of HIV

status, and the number of sex partners in heterosexual men (Fisher et al, 2006). In another study, insertive anal sex was associated with ED drug use (Purcell et al, 2005, Fisher et al, 2011) and sildenafil use showed a higher likelihood of increased sexual risk behavior (Spindler et al, 2007).

A relationship between ED drugs and other behavioral factors has been well-established in the literature. Evidence from past research suggested a positive association between ED drug use and other variables, such as recreational drug uses and sexual behaviors. However, the pattern of use between these factors over time in MSM is relatively unknown. Most studies of ED drugs in HIV and MSM have looked at the characteristics and association with ED drug use in a cross-sectional setting. Based on these studies, we can hypothesize that ED drug use will be a positively correlated over time with other behavioral factors.

Therefore, the purpose of this study was to assess the patterns of ED drug use with other factors, such as recreational drugs use, medications, as well as sexual behaviors. The average ED drug use in relation to other variables was assessed longitudinally to describe the characteristics of ED drug use in MSM and identify variables that were correlated with ED drug use across time. Furthermore, association between ED drug use and other variables within individuals was examined.

3.2 Methods

3.2.1 Study Population

Participants were from the Multicenter AIDS Cohort Study (MACS), an ongoing cohort study on MSM across 4 sites in the United States (Baltimore, Chicago, Pittsburgh, and Los Angeles) (Kaslow et al, 1987; Detels et al, 1992). Data was collected from participants during semiannual interviews and physical examinations since 1984. For the purpose of this

study, subjects were a total of 1,636 HIV positive and negative MSM who were in the MACS study from 1998. Baseline for the HIV positive group included the first visit observed by seropositive and seroconverted participants. Baseline for the HIV negative group was the first visit observed by the seronegative men. Some men had additional visits for treatment-only purposes, and these visits were omitted from the analysis.

3.2.2 Predictors

Covariates that were associated with the use of ED drugs were included as predictors in the longitudinal analysis. Demographic characteristics such as age (re-centered using the median, and re-scaled by 10 years), race/ethnicity (non-Hispanic White, non-Hispanic Black, and Other), and educational attainment (college degree, and high school or less) were included in the analysis. Smoking status was a categorical variable divided into current, former and never smokers. Alcohol consumption was categorized into four groups: binge drinker (5 or more drinks per occasion at least once per month), moderate/heavy (more than 14 drinks per week), low/moderate (1-14 drinks per week), and none (0 drinks) (Kelly et al., 2016). All predictors were self-reported, except for age, and time-varying variables. BMI was a time-varying continuous measure that was categorized into three groups according to CDC's adult BMI categories (CDC, 2017): normal (BMI<25.0), overweight (25.0≤BMI<30.0), and obese (BMI≥30.0).

3.2.3 Outcomes

The main outcome variable of interest was self-reported use of ED drugs since last visit. Prescription drugs for ED treatment, such as sildenafil, tadalafil, and vardenafil, were included in the ED drugs. Other ED drugs that were not prescribed for ED treatment were also included. This was a time-varying binary variable (yes/no).

Time-varying substance use included the self-reported use since the last visit of

marijuana, poppers (alkyl nitrites), stimulants (crack/cocaine, ecstasy, methamphetamine and uppers), heroin or other opiates, speedball (heroin and cocaine together), downers, ethyl chlorides (inhalants), GHB (gamma-Hydroxybutyric acid), injection drugs, and testosterone. For all substance use variables, all missing values were replaced with a "no" if the participants during their next observed visit reported never using the drug.

Sexual behavior variables included the number of male and female partners since last visit, and the number of partners with whom the participants engaged in protected insertive anal intercourse (PIAI) and unprotected insertive anal intercourse (UIAI) since the last visit. We transformed these variables to create three binary variables on sexual behavior: risky sex (2 or more partners vs. 0 or 1), PIAI (1 or more partners vs. 0), and UIAI (1 or more partners vs. 0). All sexual behavior variables were self-reported.

Medication variables were the use of highly active antiretroviral therapy (HAART) at the time of the visits (yes/no), self-reported depression medication use since last visit, and diabetes medication used since last visit. HCV infection status was dichotomized into positive or negative, depending on the presence of HCV antibodies or RNA. Kidney disease was dichotomized into having current or prior confirmed diagnosis versus no diagnosis. Time-varying co-morbidities assessed whether participants had pre-existing conditions, such as stroke, congestive heart failure, prostate surgery or cancer, or bladder surgery or cancer, at visits. These conditions were identified using ICD-9 codes and were selected since they were known risk factors for erectile dysfunction and contraindication for the use of erectile dysfunction drugs (Lim et al, 2002; Miranda-Sousa et al, 2006). We then dichotomized the co-morbidity variable into having at least one of the conditions or none.

3.2.3 Statistical Analysis

T-test and chi-squared tests were used to examine bivariate associations between ED

drug use at baseline and the baseline demographic characteristics, substance use, health-related variables, and sexual behaviors.

We used bivariate random intercept models (Weiss, 2005) to model pairs of response variables, one of which was ED drug use and the second was recreational drugs use, sexual behaviors, or medication use, each in turn.. This model has a parameter that describes the correlation between person-average levels of the two variables. All models adjusted for age, race/ethnicity, education, smoking, alcohol consumption, BMI, kidney disease, HCV infection, and pre-existing conditions for both variables. Output from the model includes the variances of the random intercepts and the covariance between the random intercepts from which we calculated the correlation. Significance of the correlation is the same as that for the covariance. Confidence intervals were not reported since no confidence intervals of the covariance is computed from the statistical analysis software.

To examine the association between ED drug use and response variables at particular time points within individuals, bivariate GLMM of ED drug use and the variables was fit to obtain subject-specific time-varying residuals. The residuals were then included as a predictor in the bivariate random-intercept model and the resulting sign, positive or negative, of the coefficient of the residual identified positive or negative within-subject time-varying association.

All statistical analysis was performed with SAS 9.4 (SAS Institute Inc., Cary, North Carolina).

3.3 Results

Data from 1,391 HIV positive men with 29,343 person-visits and 307 HIV negative men with 6,752 person-visits were included in the analysis. The median number of days

between visits was 182 days for both HIV positive (Interquartile range (IQR): 174-203) and

negative groups (175-196). Median visits among men in the HIV positive group was 24 (IQR:

11-29) and 25 (15-28) visits by HIV negative men (Table 1). There were 62 men who

seroconverted during the period of our study and contributed data to both HIV positive and

negative groups.

Table 2 shows descriptive statistics of ED drug users and non-users in HIV positive

subjects at baseline. The mean age was 42.1 years (SD: 8.0) and ED drug users were older

than non-users. The majority of the participants were white, had less than four-year college

degree (54.1%) and had a normal BMI (54.9%). Marijuana use was similar in both ED drug

users and non-users (39.0% vs. 41.3%) but a higher proportion of men who reported ED drug

use also reported using poppers (41.5% vs. 26.1%) and stimulants (38.6% vs. 23.0%). Half of

the subjects were on HAART at baseline with no significant difference between ED drug

users and non-users. One in ten subjects were positive for HCV infection at baseline and this

was higher in ED drug users (p=0.0027). The majority of the participants reported having

more than 2 sex partners since last visit (53.0%) and a significantly higher proportion of ED

drug users reported having more than 2 sex partners (80.3% vs. 51.3%), PIAI (67.5% vs.

41.8%) and UIAI (41.0% vs. 17.4%) since last visit (p<0.001). Similar characteristics were

observed in HIV negative subjects (Table 3). There were no significant differences between

ED drug users and non-users with substance use. A higher proportion of men who used ED

drugs reported PIAI (65.6% vs 46.3%; p=0.04), and UIAI since last visit (35.5% vs. 27.8%).

Table 4 shows the results from bivariate random-intercept models with ED drug use

and a second outcome variable in HIV positive men. Average ED drug use was weakly

correlated with average marijuana use (r=0.19), poppers (r=0.27), injection drugs (r=0.14),

and stimulants (r=0.25). GHB and heroin/opiates did not converge due to small number of

users and were not reported in the table. Depression medication use also showed a weak positive, correlation with ED drug use (r=0.08). Testosterone use showed moderate correlations with ED drug use over time (r=0.32) and similar relationships were estimated for the sexual behavior variables, including risky sex (r=0.40), PIAI (r=0.40) and UIAI (r=0.43). HIV negative subjects presented similar correlations. Average marijuana use and ED drugs were weakly correlated (r=0.19) and stimulants use with ED drugs showed slightly higher but weak correlation (r=0.22). Risky sex had a moderate relationship with using ED drugs over time (r=0.36), and PIAI (r=0.22) and UIAI (r=0.18) also showed positive correlations.

Within HIV positive individuals, marijuana (b=0.19, p<0.001), poppers (b=0.28, p<0.001), stimulants (b=0.40, p<0.001), and testosterone use (b=0.40, p<0.001) exhibited statistically significant associations with visit-level ED drug use (Table 5). Risky sex (b=0.59, p<0.001), PIAI (b=0.72, p<0.001) and UIAI (b=0.64, p<0.001) also increased with increasing ED drug use over time. In the HIV negative group, using recreational drugs were not correlated with ED usage at the visit-level within individuals. Only the sexual behavior variables, risky sex (b=0.55, p<0.001), PIAI (b=0.64, p=0.002) and UIAI (b=0.51, p=0.01), were associated with ED drug use.

3.4 Discussion

Marijuana, poppers, stimulants, and testosterone showed evidence of correlation between subject-level average use and ED drug use at a population level. Depression medication usage had a weak positively correlation with ED drug use. In addition, ED drug use and engagement in risky sexual activities, such as having more than two partners since last visit, as well as reporting protected and unprotected insertive anal intercourse, increased together over time.

These findings were consistent with other studies that examined use of ED drugs with

recreational drugs use and lifetime sexual partners (Paul et al, 2005; Halkitis and Green, 2007; Harte and Meston, 2011; Fisher et al, 2006). The results from this study supported the association between ED drug use and unprotected insertive anal intercourse from a study by Schwarcz et al. (2007). Fisher et al. (2011) noted that men who were using recreational drugs were more likely to be using ED drugs. The researchers in the same study also found an association between ED drug use and GHB and cocaine among men who were taking both Viagra and methamphetamine, while another study (Kim et al., 2002) found that users of Viagra with other drugs reported higher number of sex partners than those who did not use them together. In a descriptive analysis of users of both marijuana and ED drugs, men used ED drugs to offset their libido effect, since marijuana acts on the same cytochrome P450 enzyme as sildenafil (Eloi-Stiven et al, 2007). Furthermore, men were more likely to be engaged in unprotected insertive anal intercourse if they were using more than two drugs together (Halkitis and Parsons, 2002) and Viagra users were more likely to have increased unprotected anal sex (Swearingen and Klausner, 2005).

At an observation level within individuals, use of ED drug and other recreational drugs were positively associated on average. Testosterone, depression medication and sexual behaviors were associated with visit-level within-person ED drug use. This suggests that increased ED drug use on average was associated with increased use of recreational drugs and higher risky sex behaviors within individuals. To our knowledge, there are no studies that examined the patterns of ED drug use with other behavioral factors at an individual level. One study that investigated the longitudinal pattern of recreational drugs and high-risk sexual behavior among MSM did find that even sporadic use of drugs resulted in higher risk of engaging in unprotected anal sex (Colfax et al., 2005). Although the study did not look at ED drugs, it did suggest that some of the recreational drugs used alone contributed to risky sexual

behaviors, thereby increasing the risk of HIV transmission. Most studies that assessed the use of ED drugs, recreational drugs and risky sexual behaviors suggested that an intervention on one of the factors may not be enough to help reduce the transmission. Our study supports a similar idea since the concurrent use of ED drugs and recreational drugs, as well as risky sexual behaviors, were all positively associated over time.

The strengths of our study include the use of a large, well-defined cohort data. Most prior studies were of cross-sectional designs, or only looked at baseline characteristics, and there was a lack of studies examining ED drug use in a longitudinal setting. We were able to assess ED drug use longitudinally from the MACS data set, which included socially sensitive data collected through a standardized method for data collection. The statistical method used in our analysis has several advantages. The bivariate random-intercept model shows whether average person-level usage of two variables are correlated, as well as whether these variables move up and down together at the visit-level within individuals. Joint modeling of the two variables as a bivariate response that was repeatedly measured over time allows for such analysis. In addition, variables with different numbers of observations or different times of observation can be analyzed by this model.

The results from the study have several limitations. We did not look at the frequency or dosage information of ED drugs as they were not available. Different types of ED drugs were also not examined for the same reason. Prescription information for drugs was not available for subject in the MACS, so the ED drug use of participants could not be linked to confirm their self-reported responses. Social demographic information, as well as recreational drugs and sexual activities were also from self-reported data. Even though the use of ACASI helps participants respond to sensitive questions more accurately, there can still be misreporting of information. Finally, partner's HIV status was unknown and pre-exposure

prophylaxis (PrEP), which was approved by the FDA in 2012, was unavailable for most of our study period.

3.4 Public Health Implications

Our study showed a positive relationship between ED drug use with other outcomes, such as marijuana, poppers and UIAI. The results suggest a positive association between the use of ED drugs over time and other recreational drugs. High-risk sexual behaviors also increased with average ED drug use.

It is unsafe to administer ED drugs with nitrates/nitrites and protease inhibitors, as concurrent use of Viagra and nitrite inhalers (poppers) could lead to hypotension. In addition, HIV infected individuals using protease inhibitors are recommended to use a lower dose of Viagra (James, 1998; Lim et al, 2002). This study showed that participants were taking poppers, or were on HAART, while using ED drugs. Therefore, monitoring the use of these drugs may be useful to inform the planning and promotion of interventions for healthy behaviors. Men taking ED drugs need to be informed on drug interactions and safe sex behaviors (Chu et al, 2003; Kim et al, 2002). Viagra usage alone was associated with the risk of seroconversion (Ostrow et al, 2011), and the use in combination with recreational drugs and risky sexual behaviors may have detrimental effects in the MSM population. As researchers have proposed in the past (Chu et al., 2003; Swearingen and Klausner, 2005), we many need to provide better suggestions or stronger public messages to men who use these drugs and will need to extend it to those who obtain ED drugs from other sources for recreational use. With aging, the use of ED drugs will increase among sexually active men, and intervention strategies may be needed to prevent HIV transmission.

3.5 Conclusion

This study showed that use of ED drugs was positively correlated with use of other recreational drugs and this also occurred at the visit-level within individuals over time. In addition, both HIV positive and negative MSM were more likely to engage in risky sexual behaviors over time with increased average ED drug use. Currently, it is unclear to what extent ED drugs affect the immune system. Future research is needed to understand the effects of ED drugs on immune cells and markers of inflammation in MSM.

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TABLES AND FIGURES

Table 1: Median number of visits and days between each visit by MACS participants during 1998-2016

	Number of visits				Average days between visits				
	N	Median	Q1	Q3	Mean	SD	Median	Q1	Q3
HIV Positive	1,391	24	11	29	203.6	166.2	182	174	203
HIV Negative	307	25	15	28	191.8	107.2	182	175	196

Q1: 25th percentile. Q3: 75th percentile.
Note: Participants who seroconverted were included in HIV positive group

Table 2: Demographic characteristics of HIV positive MACS participants by baseline ED drug use since last visit

Variable	ED Drug Use				Total		p*
	No		Yes				
	n=1,307 (94.0%)		n=84 (6.0%)		N=1,391 (100%)		
Age (mean±SD)	41.9 ± 7.8		44.6 ± 9.6		42.1 ± 8.0		0.001
Race							0.67
White	740	(56.6)	47	(56.0)	787	(56.6)	
Black	358	(27.4)	26	(31.0)	384	(27.6)	
Other	209	(16.0)	11	(13.1)	220	(15.8)	
Education (college or higher)	528	(46.0)	38	(45.2)	566	(45.9)	0.90
Smoking status							0.22
Current	508	(39.3)	40	(48.8)	548	(39.9)	
Former	417	(32.3)	21	(25.6)	438	(31.9)	
Never	367	(28.4)	21	(25.6)	388	(28.2)	
Alcohol consumption							0.09
Binge	150	(11.7)	12	(14.6)	162	(11.8)	
Moderate/Heavy	287	(22.3)	21	(25.6)	308	(22.5)	
Low/Moderate	596	(46.3)	42	(51.2)	638	(46.6)	
None	255	(19.8)	7	(8.5)	262	(19.1)	
BMI (kg/m^2)							0.03
Obese (≥30)	136	(10.8)	2	(2.4)	138	(10.3)	
Overweight (25-29.9)	431	(34.2)	36	(43.4)	467	(34.8)	
Normal (≤24.9)	692	(55.0)	45	(54.2)	737	(54.9)	
Study site							0.02
Baltimore	305	(23.3)	30	(35.7)	335	(24.1)	
Chicago	307	(23.5)	11	(13.1)	318	(22.9)	
Pittsburgh	256	(19.6)	19	(22.6)	275	(19.8)	
Los Angeles	439	(33.6)	24	(28.6)	463	(33.3)	
Marijuana	529	(41.3)	3232	(39.0)	561	(41.2)	0.69
Poppers	335	(26.1)	34	(41.5)	369	(27.1)	<0.001
Stimulants†	296	(23.0)	32	(38.6)	328	(23.9)	0.001
Heroin/opiates	40	(3.1)	2	(2.5)	42	(3.1)	0.99
Speedball	18	(1.4)	2	(2.4)	20	(1.5)	0.34
Ethyl chloride	4	(0.3)	1	(1.3)	5	(0.4)	0.26
GHB	9	(0.7)	6	(7.5)	15	(1.1)	<0.001
Injection drugs use	90	(7.1)	16	(19.5)	106	(7.8)	<0.001
Diabetes medication	34	(2.6)	1	(1.2)	35	(2.5)	0.72
Depression medication	326	(24.9)	27	(32.1)	353	(25.4)	0.14
Kidney disease	4	(0.3)	1	(1.2)	5	(0.4)	0.27
Testosterone	24	(1.8)	7	(8.3)	31	(2.2)	0.002
HAART	695	(53.2)	36	(42.9)	731	(52.6)	0.07
HCV infection	129	(9.9)	17	(20.2)	146	(10.5)	0.003
Pre-existing conditions‡	70	(5.4)	5	(6.0)	75	(5.4)	0.80
Risky Sex (≥2 partners)	656	(51.3)	65	(80.3)	721	(53.0)	<0.001
PIAI (≥1 partner)	529	(41.8)	54	(67.5)	583	(43.3)	<0.001
UIAI (≥1 partner)	220	(17.4)	32	(41.0)	252	(18.8)	<0.001

All results are in N(%) unless otherwise stated.
Statistically significant results are in bold.
*P-values were computed using t-test and chi-squared tests for bivaritate association between ED drug use and the covariates.
†Cocaine, ecstasy, methamphetamine, uppers.
‡Stroke, coronary heart failure, prostate surgery/cancer, bladder surgery/cancer.

Table 3: Demographic characteristics of HIV negative MACS participants by baseline ED drug use since last visit

Variable	ED Drug Use				Total		p*
	No		Yes				
	n=274 (89.3%)		n=33 (10.7%)		N=307 (100.0%)		
Age (mean ± SD)	43.9 ± 9.6		47.8 ± 9.7		44.4 ± 9.6		0.03
Race							0.43
White	148	(54.0)	19	(57.6)	167	(54.4)	
Black	100	(36.5)	9	(27.3)	109	(35.5)	
Other	26	(9.5)	5	(15.2)	31	(10.1)	
Education (college or higher)	139	(51.1)	12	(36.4)	151	(49.5)	0.11
Smoking status							0.07
Current	119	(43.6)	10	(30.3)	129	(42.2)	
Former	79	(28.9)	16	(48.5)	95	(31.1)	
Never	75	(27.5)	7	(21.2)	82	(26.8)	
Alcohol consumption							0.18
Binge	39	(14.3)	3	(9.1)	42	(13.7)	
Moderate/Heavy	69	(25.3)	6	(18.2)	75	(24.5)	
Low/Moderate	119	(43.6)	21	(63.6)	140	(45.8)	
None	46	(16.9)	3	(9.1)	49	(16.0)	
BMI (kg/m^2)							0.30
Obese (≥30)	54	(19.7)	3	(9.1)	57	(18.6)	
Overweight (25-29.9)	98	(35.8)	12	(36.4)	110	(35.8)	
Normal (≤24.9)	122	(44.5)	18	(54.6)	140	(45.6)	
Study site							0.23
Baltimore	61	(22.3)	12	(36.4)	73	(23.8)	
Chicago	62	(22.6)	8	(24.2)	70	(22.8)	
Pittsburgh	89	(32.5)	9	(27.3)	98	(31.9)	
Los Angeles	62	(22.6)	4	(12.1)	66	(21.5)	
Marijuana	98	(35.9)	15	(45.5)	113	(36.9)	0.28
Poppers	59	(21.6)	12	(36.4)	71	(23.2)	0.06
Stimulants*	65	(23.8)	13	(39.4)	78	(25.5)	0.05
Heroin/opiates	21	(7.7)	3	(9.1)	24	(7.9)	0.73
Speedball	13	(4.8)	1	(3.0)	14	(4.6)	1.00
Ethyl chloride	1	(0.4)	0	(0.0)	1	(0.3)	1.00
GHB	1	(0.4)	1	(3.0)	2	(0.7)	0.001
Injection drugs use	44	(16.2)	6	(18.2)	50	(16.4)	0.77
Diabetes medication	7	(2.6)	3	(9.1)	10	(3.3)	0.08
Depression medication	49	(17.9)	8	(24.2)	57	(18.6)	0.37
Kidney disease							
Testosterone							
HAART							
HCV infection	55	(20.1)	7	(21.2)	62	(20.2)	0.88
Pre-existing conditions†	14	(5.1)	3	(9.1)	17	(5.5)	0.41
Risky Sex (≥2 partners)	173	(63.4)	26	(78.8)	199	(65.0)	0.08
PIAI (≥1 partner)	125	(46.3)	21	(65.6)	146	(48.3)	0.04
UIAI (≥1 partner)	75	(27.8)	11	(35.5)	86	(28.6)	0.37

All results are in N(%) unless otherwise stated.
Statistically significant results are in bold.
*P-values were computed using t-test and chi-squared tests for bivaritate association between ED drug use and the covariates.
†Cocaine, ecstasy, methamphetamine, uppers.
‡Stroke, coronary heart failure, prostate surgery/cancer, bladder surgery/cancer.

Table 4: Generalized linear mixed models* with ED drug use and a second outcome variable using bivariate random-intercept model in HIV positive and negative participants.

Outcome Variable	HIV positive		Outcome Variable	HIV negative	
	r	p		r	p
Marijuana	0.19	<0.001	**Marijuana**	0.19	0.005
Poppers	0.27	<0.001			
Stimulants	0.25	<0.001	**Stimulants**	0.22	0.002
Testosterone	0.32	<0.001			
Depression medication	0.08	0.02	Diabetes medication	-0.06	0.42
HAART	0.03	0.37			
Risky sex	0.41	<0.001	**Risky sex**	0.36	<0.001
PIAI	0.40	<0.001	**PIAI**	0.22	0.002
UIAI	0.43	<0.001	**UIAI**	0.18	0.01

* Models were adjusted for age, race, education, smoking status, alcohol consumption, BMI, kidney disease, HCV infection, pre-existing conditions

Note: Models that did not converge are not presented in the table. Standard error (confidence intervals) are not given since it could not be computed from the software.

Table 5: Generalized linear mixed model using ED drug residuals* and outcome variable in a bivariate random-intercept model for associations at an individual level across time

Outcome Variable	HIV positive			HIV negative		
	Estimate	SE	p	Estimate	SE	p
Marijuana	**0.19**	0.05	<0.001	0.16	0.12	0.18
Poppers	**0.28**	0.05	<0.001			
Stimulants	**0.40**	0.06	<0.001	0.13	0.17	0.45
Testosterone	**0.40**	0.06	<0.001			
Depression medication	**0.17**	0.05	<0.001			
Diabetes medication				0.15	0.18	0.40
HAART	-0.02	0.06	0.78			
Risky sex	**0.59**	0.05	<0.001	**0.55**	0.11	<0.001
PIAI	**0.72**	0.05	<0.001	**0.64**	0.12	<0.001
UIAI	**0.64**	0.05	<0.001	**0.51**	0.13	<0.001

*Residual = (Observed proportion of ED drug use – Expected proportion of ED drug use). Computed from generalized linear mixed models adjusted for age, race, education, smoking status, alcohol consumption, BMI, HIV status, kidney disease, HCV infection, pre-existing conditions.
Statistically significant results are set in bold.

CHAPTER 4

Manuscript #2

Effect of Erectile Dysfunction drugs on the CD4 and CD8 T-cells in men who have sex with men in the Multicenter AIDS Cohort Study: Using G-computation

Abstract

Background: Erectile dysfunctions drugs are commonly used in men who have sex with men as both prescription drugs and recreational purposes. However, the effects of these drugs on the immune system are still unclear.

Methods: A total of 1,391 HIV positive subjects and 307 HIV negative subjects were included from the Multicenter AIDS Cohort Study from 1998 onwards. We applied longitudinal marginal structural models to assess the causal relationship between erectile dysfunction drugs and CD4 and CD8 T cells.

Results: ED drug use over time increased the number of CD4 cells in HIV positive men but decreased CD4 counts in the negative men. The causal mean difference in CD4 count in HIV positive men after 1 year of erectile dysfunction drug use was 57.6 cells/μL and increased to 117.7 cells/μL after 10 years. CD8 cell counts were higher among users over a 10-year period compared to non-users in the HIV positive group but showed almost no significant differences in HIV negative group. CD4 percentage constantly remained higher among erectile dysfunction drug users in the HIV positive men.

Conclusion: Erectile dysfunction drugs appear to significantly improve CD4 cells counts and CD4 percentages in HIV positive men who have sex with men. This may suggest a favorable immunomodulatory effect of erectile dysfunction drugs.

4.1. Background

Erectile dysfunction (ED) drugs are commonly used in men having sex with men (MSM) population for the treatment of ED and as a recreational drug (*Harte and Meston, 2011; Kim et al., 2002*) and among older men (*Fisher et al., 2006*). Recreational uses of ED drugs are associated with other drug uses, such as marijuana and cocaine, as well as risky sexual activities (*Fisher et al., 2006; Purcell et al., 2005*). ED drugs, such as sildenafil, are phosphodiesterase 5 (PDE5) inhibitors. It controls the level of cyclic guanosine monophosphate (cGMP) by preventing the degradation of cGMP to GMP. The inhibition of cGMP degradation then acts to regulate the levels and activities of immune cells (*Kniotek and Boguska, 2017*).

Despite the frequent usage of ED drugs, the effect on the human immune system is still unclear. There are only a few studies that have examined the effect of ED drugs on the immune system. In animal studies, ED drugs, mainly sildenafil, have shown immunomodulatory effects in healthy mice (*Karakhanova et al., 2013; Szczypka and Obminska-Mrukowicz, 2010*) and in another study showed increased survival of tumor-bearing mice when sildenafil was applied (*Serafini et al., 2006*). In one study that reported the effect of sildenafil on the human immune cells in vitro also showed the immunomodulatory effects on healthy human lymphocytes (*Pifarre et al, 2014*). Recently, the direct effect of sildenafil on cells have been shown to involve cGMP and Na^+/K^+-ATPase activity which prevents inflammation development (*Nunes et al., 2016*).

Hence, there is some evidence of the potential immunomodulatory effects of ED drugs in the human immune system. However, the longitudinal effect of ED drugs on the immune system in the MSM population has not been documented. Therefore, this study aimed to examine the effect of ED drugs on CD4 and CD8 T cells over time in MSMs. Based on existing literature,

it was hypothesized that ED drug users would have higher CD4 cell counts and improved immune capacity over time compared to non-ED drug users.

4.2 Methods

4.2.1 Study Population

The participants were from the Multicenter AIDS Cohort Study (MACS), an ongoing cohort study on MSM with semi-annual study visits from 4 study sites in the United States (Baltimore, Chicago, Pittsburgh, Los Angeles) (*Detels et al., 1992*). Data from a total of 1636 HIV positive and negative men, were used in the analyses (age: 19-70 years). Subjects with only one visit in the study were excluded from analysis. In addition, clinical visits for treatment purposes were also omitted from the analysis. Subjects who were in the study from 1998 were included in the study since ED drugs were approved by the FDA in the market in 1998.

4.2.2 Measures

The exposure variable was self-reported ED drug use since the last visit. Known ED drugs such as sildenafil, tadalafil and vardenafil including self-reported use of these drugs that were not prescribed for ED was also included. Thus, the ED drug use included both prescribed and recreational use.

Covariates included in the study were demographic characteristics such as age, which was computed using date of birth and date of visit, race (white vs. other) and education level (college degree vs. less than college). Smoking status (smoker vs. non-smoker) and alcohol consumption (binge/heavy vs. moderate/light vs. non-drinker) were self-reported and obesity (obese/overweight vs. normal) used BMI cut-offs of greater than 25. Other confounding variables included substance use (marijuana, poppers, stimulants, testosterone), medication

(HAART, depression medication), and health-related factors such as HCV infection, and other co-morbidities (stroke, congestive heart problems, prostate cancer/surgery, bladder cancer/surgery) using ICD-9 codes. Sexual behavior variables included the number of men with whom the subjects engaged in unprotective insertive anal intercourse since the last visit (1 or more vs. 0), and number of male and female sex partners (2 or more vs. 0/1). Insertive anal intercourse was used since these would be the men who would use ED drugs. All variables except age, BMI and viral load were self-reported.

CD4 and CD8 cell counts from previous visit were included as confounding variables in the analysis of CD4 and CD8 cells, respectively. Viral load was used as a continuous variable. CD4 and CD8 T cells were measured from blood samples taken during study visits. Viral load, CD4 and CD8 T cells were log-transformed.

4.2.3 Statistical Analysis

Subjects in the study were divided into two groups according to their HIV status (positive/negative). Descriptive statistics were used to show the baseline characteristics of subjects. Baseline was defined as the first visit made by participants after 1998 when the first ED drugs were available on the market. Baseline for the positive group included the first visit of HIV positive subjects in the study as well as the subjects who seroconverted during the follow-up of the study.

The MACS data is a complex longitudinal data set that includes time-varying exposure, confounders and outcome. Therefore, to estimate the causal effect of ED drugs on CD4 and CD8 cell counts, we implemented g-computation (*Robins, 1986; Robins, 2000*) in a longitudinal setting shown in the directed acyclic graph (DAG) in Figure 1. Time-varying confounding variables (L_t) in such longitudinal settings included marijuana, poppers, stimulants, testosterone, and depression medication use, as well as the number of sex partners

and number of UIAI partners. These variables had to be adjusted for later exposures, (A_t), but were also on the indirect path from previous exposure (A_{t-1}) to the later outcome (Y_t), in which case they should not be adjusted for in the analysis (*Hernán et al., 2000; Robins et al., 2000; Hernán et al., 2001*). By using marginal structural models, it was possible to eliminate such time-varying confounding effects while preserving the causal intermediate paths. Figure 2 shows the DAG after intervention on exposure at each time point. Here, confounding effects by the time-dependent variables are eliminated while the intermediate pathways from previous exposure to the next outcome remain.

If we let A_t be exposure, Y_t be outcome, L_t a set of covariates (confounding variables) where t represents time/visit ($t=1, 2,…, 21$), Y_a is the potential outcome of Y when A is set to a and *newA* is when we set A to be a. Firstly, we specified and fitted the models for the outcome of interest, $E(Y_t|A_t, L_t, Y_{t-1})$, and the models for time-varying confounders, L's: $P(L_t| A_{t-1}, L_{t-1})$, using the observed Y's, A's, and L's and saved the regression coefficients. Next, we created two copies of the data, where one was given *newA*=1 (all subjects took ED drugs) and the other was given *newA*=0 (all subjects did not take ED drugs). Then, using Markov Chain Monte Carlo simulation with 1,000 repetitions, we created *newL*'s and new potential outcomes, $E(Y_a|newA, newL)$, in each time point using the saved parameters. Finally, we regressed Y_a on *newA* to estimate the causal mean difference. Confidence intervals were computed by summarizing the results over the repetitions using the 2.5th and 97.5th percentile as the lower and upper confidence limits. This study included multiple A's, L's and Y's over time, so construction of new variables was repeated for each time point to account for these extra terms. Interactions between ED drug use and time-fixed variables, such as age, race and education were included in the model. Interactions terms between ED drugs and other confounding variables were tested but showed no statistical significance and were not

included. Variables included in the analysis are shown in Table 1.

Observations from previous visits were carried forward and used to replace missing values for the variables that were "used since last visit" and for which the "ever used" values did not change during the visits. Such variables included smoking, alcohol consumption, obesity, HCV infection and comorbidities. Data were analyzed in SAS 9.4 (SAS Institute Inc., Cary, North Carolina).

4.3 Results

Data was available for 1,636 men from the MACS study with 36,095 observations. Some men in the study contributed to both the HIV positive and negative groups, so there were 1,391 HIV positive men with 29,343 observations, and 307 HIV negative men with 6,752 observations. Overall, the study participants made an average of 22.1±10.8 visits, with 21.1±11.3 visits by HIV positive and 22.0±9.7 visits by HIV negative men (Table 2). Table 3 shows the descriptive baseline statistics of ED drug users and non-users among the subjects that were included in the analyses. At baseline, 9.6% of HIV negative men reported ED drug use compared to 6.0% of HIV positive men. The mean age of HIV positive men was 42.5±7.8 years and 44.8±9.5 among HIV negative, with a higher mean age among ED drug users compared to non-users (HIV positive: 43.5 vs. 42.4 years; HIV negative: 47.7 vs. 44.5 years). The majority of the participants in both groups was white, non-smokers, and had normal BMI. Among HIV positive men, 45.7% of ED drug users and 54.2% of non-users were on HAART at baseline. HCV infection was positive in 18.5% of HIV positive and 27.6% of HIV negative men who reported ED drug use at baseline. Both groups of men had a similar proportion of at least one pre-existing condition (HIV positive: 5.7%; HIV negative: 5.6%). The majority in both groups who had taken ED drugs reported having more than 2 sex partners (HIV positive: 70.4%; HIV negative: 69.0%), while 42.0% of ED drug users in HIV positive and 51.7% in

HIV negative men were engaged in unprotected insertive anal intercourse at baseline. CD4 cell counts at baseline for HIV positive men among ED drug users and non-users were 553.0±32.9 cells/µL and 495.6±8.1 cells/µL, respectively. In HIV negative men the CD4 cell counts were 961.1±300.2 cells/µL and 967.3±319.0 cells/µL for user and non-users, respectively. ED drug users had similar CD4 percentages for both groups. CD8 cell counts among HIV positive subjects were higher among non-users while ED drug users in the HIV negative group had higher CD8 counts. The CD4:CD8 cell ratio was close to 0.5 for HIV positive men (ED drug users: 0.56±0.36; non-users: 0.66±0.40) and was 2 for the negative group (ED drug users: 2.01±0.97; non-users: 1.92±0.97).

Table 4 shows the causal mean differences (MD) comparing ED drug users and non-users in the outcome for 5 time points: Year 0 (baseline), 1, 2, 5 and 10. The mean CD4 cell count in the HIV positive group was higher in ED drug users at baseline compared to the non-users. The causal MD was 107.3 (95% CI: 73.3–141.5) and this was slightly decreased as shown by the MD in year 1 with 57.6 (95% CI: 17.8–94.8). This difference was reduced further by year 2 with 56.4 (15.2–98.3) and year 5 with 28.0 (-21.6–85.0) but gradually increased again with a difference of 117.7 (37.1–197.4) after 10 years. The causal MD's for HIV negative group were -43.7 (-91.9–3.0), -57.7 (-111.6–-4.3), 51.7 (2.7–100.9), -74.5 (-132.6–-19.7) and -55.9 (-106.7–-4.6) for the five time points. When CD8 cell counts was the outcome, the causal MD's were -23.1 (-60.4–12.4), -7.2 (-50.6–34.2), -60.0 (-104.4–-15.5), 16.3 (-46.7–76.1) and 95.3 (18.7–169.5) for HIV positive and -29.4 (-69.2–10.8), -30.0 (-68.1–10.6), 22.3 (-17.2–61.0), -0.6 (-45.6–46.1) and 47.0 (-2.7–105.1) for HIV negative men. CD4 percentage was higher at baseline among ED drug users compared to non-ED drug users for HIV positive group and remained higher over time. In the HIV negative group, CD4 percentage showed no difference between ED drug users and non-users until later. The average differences in the

CD4:CD8 cell ratio remained constant over time in the positive group, whereas in the negative group, the differences gradually reduced so that non-users had a higher ratio than the ED drug users.

Figure 3 illustrates the causal mean differences with 95% CI for all outcomes at each time interval. This shows clearly the mean differences for each time point comparing ED drug users and non-users for both HIV groups. Over time, HIV positive ED drug users have higher CD4 counts, percentages and CD4:CD8 ratios compared to non-users. HIV negative ED drug users have lower CD4, percentage and ratios over time. Figure 4 shows how the mean outcomes among ED drug users and non-users change over time. The differences between the two lines at each time point in Figure 4 reflect the causal mean differences in Figure 3.

4.4 Discussion

In this study using the MACS data and applying a causal inference method, ED drugs were causally related with differences in CD4 and CD8 T cells in both HIV positive and negative groups. ED drug use over time increased the number of CD4 cells in HIV positive men but decreased CD4 counts in the negative men. CD8 cell counts were higher among ED drug users over 10 years of use compared to non-users in the HIV positive group but showed almost no significant differences in HIV negative group. However, the CD8 counts were lower for the first 4 years among ED drug users than non-users in the HIV positive group. The CD4 cells as a percentage of total leukocytes and the CD4:CD8 ratio reflected these results. The CD4:CD8 ratio generally stayed constant over time but was higher among users in the positive group and lower in the negative group.

The results from this study must be carefully interpreted since they deal with counterfactuals from marginal structural models. These provide unbiased estimates of the causal mean difference in CD4 and CD8 cells between ED drug user and non-users. Thus, the causal MD

in CD4 cell counts after 1 year is 57.6 (95% CI: 17.8–94.8), which means that men who, contrary to fact, would have self-reported taking the ED drugs at each visit for one year would have had a higher CD4 count, with an average difference of 57.6 cells/µL, compared to men who would have reported not taking the ED drugs during visits in the first year.

The findings from this study were similar to results shown in previous studies on the effect of ED drugs on CD4 and CD8 cells in animal studies. Naïve CD4 cells were found to be significantly higher and central memory CD8 cells were lower when healthy male mice were treated with sildenafil (*Karakhanova et al., 2013*). Another study in cells isolated from mice showed oral administration of sildenafil resulted in increased CD8 cells (*Szczypka and Obminska-Mrukowicz, 2010*). An in vitro study of sildenafil-treated mice showed decreased adaptive immune responses while T regulatory cell functions were enhanced (*Pifarre et al., 2014*). Two other studies (*Califano et al., 2015*; *Weed et al., 2015*) have also shown that tadalafil enhanced systemic immunity by increasing T-cell proliferation, both CD4 and CD8 T-cells, as well as inhibiting immunosuppressive function by suppressing inducible NOS and arginase-1 (inhibits immunosuppressive function on MDSC's).

CD4 lymphocyte percentages and the CD4:CD8 ratios are useful markers for monitoring individuals with HIV (*Taylor et al., 1989*). The normal range of CD4:CD8 ratio among healthy individuals is considered to be between 1.5 and 2.5 (*McBride et al., 2017*). Among the HIV negative men in this study, both ED drug users and non-users had ratios between 1.5 and 2.0 but users were lower. This was expected considering the CD8 cell counts were higher among the users while CD4 remained within a similar range over time. Among HIV positive men, the CD4:CD8 ratios were below 1 but ED drug users had higher ratios than non-users.

CD8 T cells were initially slightly lower among ED drug users for both HIV positive and negative men, but after continuous use, the CD8 T cell counts were higher compared to non-

users. Several factors may explain the changes in the levels of CD8 cells including viral infections and HAART (*Cao, et al., 2016*). Another possible explanation may be the restoration of CD8 cells by sildenafil. Meyera et al. (2011) showed, in vivo, that sildenafil partially restored T-cell receptor (TCR ζ-chain expression) in tumor-bearing mice. In addition, Serafini et al. (2006) also showed greatly increased CD8 cells with sildenafil in mice with tumors.

There are several limitations to this study. The present study did not look at the frequency or dosage of ED drugs as they were not available. Separate analyses for different types of ED drugs were also not examined. It was not possible to distinguish between the drugs as there were insufficient data on types of drug used during each visit. Prescription data for drugs was not available for each subject, and so the ED drug use could not be linked to confirm their self-reported responses. Social demographic information, as well as recreational drugs and sexual activities were also from self-reported data. Even though the use of ACASI helped participants respond to sensitive questions more accurately, there can still be misreporting of information. Partner status was unknown and pre-exposure prophylaxis (PrEP) was not included in the analysis as it was only made available by the FDA in 2012, and was not available for the majority of the study period. Finally, our results cannot be compared directly with other studies since the analyses in this study used marginal structural models. Moreover, the outcome models must be correctly specified and assumptions on consistency, positivity and no unmeasured confounding must be made after controlling for major confounders of ED drug effects and CD4 and CD8 cell outcomes (*Hernán and Robins, 2019*).

To our knowledge, this is the first study that examined the use of ED drugs over time in humans, and in MSMs. Most of the prior evidence was from animal experiments, and there was a lack of studies looking at the longitudinal use of ED drugs in humans. The strengths of

this study included the use of data from a well-studied, large cohort of MSM population in the U.S. This study was able to examine the effect of ED drugs on the human immune cells over a 10-year period and included important subpopulations and socially sensitive data collected through a standardized method for data collection. In addition, both HIV positive and negative MSMs were included in the analysis. The marginal structural model method used in the analyses has several advantages. G-computation produces efficient estimates with small standard errors and is able to deal with time-varying exposure and confounding (*Daniel et al., 2013; Wang et al., 2017*).

4.5 Implications

The results from this longitudinal data provide information about how ED drugs affect the level of immune capacity in MSM populations. As most developed countries including the United States have an aging population, as is the MSM population. Estimation of MSM population in the U.S. showed the 35 to 54 age group to have the highest rate of MSM living with HIV (*Purcell et al., 2012*). Consequently, the prevalence of ED and ED drug use will most likely increase. Hence, understanding the effects of ED drugs on the immune cells is important.

The effects of ED drugs in MSMs that resulted in higher CD4 counts and percentages, especially in HIV positive men, may have some clinical implications. Residual inflammation after successful treatment with anti-retroviral therapy in men with HIV has been a problem (*Massanella, et al., 2017*). If ED drugs increase levels of CD4 cells and enhance the immune capacity in MSMs, use of ED drugs may not only be limited to ED treatment, but may also be applicable to other agents suppressing the immune response.

4.6 Conclusion

The data from this study suggested that men who took ED drugs over time resulted in higher CD4 cell counts compared to men who were non-users. CD8 cell counts were slightly lower among users at first, but over time increased. We believe this study has contributed to the growing body of evidence showing the favorable immunomodulatory effects of ED drugs. Future studies should be performed to investigate the effect of different ED drugs in various phenotypes of T cells and other innate immune cells.

TABLES AND FIGURES

Table 1: Summary of variables used in the g-computation analysis.

Outcome model (Y)	Time-varying confounder model (L)
ED drug	ED drug[*]
Age	Age[*]
Race	Race[*]
Education level	Education level[*]
Smoking	Smoking[*]
Alcohol consumption	Alcohol consumption[*]
Obesity	Obesity[*]
Marijuana	Marijuana[*]
Poppers	Poppers[*]
Stimulants	Stimulants[*]
Testosterone[†]	Testosterone[*†]
Depression medication	Depression medication[*]
HAART[†]	HAART[*†]
HCV infection	HCV infection[*]
Co-morbidities	Co-morbidities[*]
Unprotected insertive anal intercourse	Unprotected insertive anal intercourse[*]
Number of sex partners	Number of sex partners[*]
Viral load[†]	
Previous outcome[*]	

[*]Lagged variable (previous visit).

[†]Variable not included in the analyses of HIV negative subjects.

Table 2. Average number visits and days since last visit among study subjects

	Number of visits			Days between visits		
	N	Mean	SD	N[*]	Mean	SD
Total[†]	1,636	22.1	10.8	36,095	201.8	156.7
HIV Positive	1,391	21.1	11.3	29,343	203.6	166.2
HIV Negative	307	22.0	9.7	6,752	191.8	107.2

*Total number of observations in the study.

†HIV positive and negative men do not add up to the total number since some negative men seroconverted during the study period, contributing to both groups.

Table 3. Descriptive baseline characteristics among HIV positive and negative men in the study who reported ED drug use since last visit compared to those who did not report ED drug use since last visit.

| | Erectile Dysfunction Drug Use | | | | | Erectile Dysfunction Drug Use | | | |
| | No (n=1,230) | | Yes (n=81) | | | No (n=273) | | Yes (n=29) | |
HIV Positive	N	(%)	N	(%)	HIV Negative	N	(%)	N	(%)
Age[*]	42.4	± 7.69	43.5	± 9.24	Age[*]	44.5	± 9.39	47.7	± 10.20
Non-white	509	(41.4)	42	(51.9)	Non-white	125	(45.8)	13	(44.8)
Education (College or higher)[§]	636	(51.7)	30	(37.0)	Education (College or higher)	139	(50.9)	15	(51.7)
Smoker	461	(37.5)	36	(44.4)	Smoker	122	(44.7)	14	(48.3)
Alcohol consumption					Alcohol consumption				
Binge/Heavy	107	(8.7)	12	(14.8)	Binge/Heavy	29	(10.6)	2	(6.9)
Low/Moderate	262	(21.3)	18	(22.2)	Low/Moderate	72	(26.4)	7	(24.1)
Obesity/Overweight (BMI>25)	124	(10.1)	6	(7.4)	Obesity/Overweight (BMI>25)	50	(18.3)	3	(10.3)
Marijuana	505	(41.1)	40	(49.4)	Marijuana	86	(31.5)	15	(51.7)
Poppers	320	(26.0)	29	(35.8)	Poppers	58	(21.3)	8	(27.6)
Stimulants†[§]	270	(22.0)	35	(43.2)	Stimulants†	66	(24.2)	10	(34.5)
Depression medication[§]	300	(24.4)	32	(39.5)	Depression medication	49	(18.0)	1	(3.5)
Testosterone	18	(1.5)	3	(3.7)					
HAART	666	(54.2)	37	(45.7)					
HCV infection[§]	118	(9.6)	15	(18.5)	HCV infection	54	(19.8)	8	(27.6)
Pre-existing conditions‡	72	(5.9)	3	(3.7)	Pre-existing conditions‡	14	(5.1)	3	(10.3)
Sex partners (≥2 partners)[§]	497	(40.4)	57	(70.4)	Sex partners (≥2 partners)	140	(51.3)	20	(69.0)
UIAI (≥1 partner)[§]	213	(17.3)	34	(42.0)	UIAI (≥1 partner)	62	(22.7)	15	(51.7)
Viral load[*]	32609.7	± 112124	43141.8	± 119050					
CD4 cell count[*]	495.6	± 8.1	553.4	± 32.9	CD4 cell count[*]	967.3	± 319.0	961.1	± 300.2
CD4 %[*§]	25.4	± 11.2	27.8	± 10.5	CD4 %[*]	47.2	± 8.58	47.7	± 8.78
CD8 cell count[*]	1003.2	± 489.0	997.2	± 623.7	CD8 cell count[*]	532.8	± 340.5	578.1	± 187.7
CD4:CD8 ratio[*§]	0.56	± 0.36	0.66	± 0.40	CD4:CD8 ratio[*]	1.92	± 0.79	2.01	± 0.97

[*] All continuous variables are expressed as: mean ± s.d.

† Include cocaine, ecstasy, methamphetamine, and uppers.

‡ Include stroke, coronary heart failure, prostate surgery/cancer, bladder surgery/cancer.

§ $p < 0.05$.

BMI: Body Mass Index. HAART: Highly Active Antiretroviral Therapy. HCV: Hepatitis C Virus. UIAI: Unprotected insertive anal intercourse.

Table 4. Causal mean differences (MD) and 95% confidence intervals calculated by g-computation for CD4 and CD8 cell outcomes in ED drug users and non-users among HIV positive and negative men at time points 0, 1, 2, 5 and 10 years of follow-up.

	Year	HIV Positive			HIV Negative		
		MD	95% CI		MD	95% CI	
CD4 count	0	**107.3**	**73.3** ,	**141.5**	-43.7	-91.9 ,	3.0
	1	**57.6**	**17.8** ,	**94.8**	**-57.7**	**-111.6** ,	**-4.3**
	2	**56.4**	**15.2** ,	**98.3**	**51.7**	**2.7** ,	**100.9**
	5	28.0	-21.6 ,	85.0	**-74.5**	**-132.6** ,	**-19.7**
	10	**117.7**	**37.1** ,	**197.4**	**-55.9**	**-106.7** ,	**-4.6**
CD4 %	0	**3.8**	**2.7** ,	**4.9**	0.0	-1.3 ,	1.3
	1	**3.3**	**1.8** ,	**4.6**	-0.6	-1.8 ,	0.6
	2	**4.1**	**2.6** ,	**5.6**	0.0	-1.1 ,	1.2
	5	**3.0**	**0.9** ,	**4.9**	**-2.3**	**-3.6** ,	**-0.9**
	10	**3.1**	**0.3** ,	**5.7**	**-3.3**	**-4.7** ,	**-1.8**
CD8 count	0	-23.1	-60.4 ,	12.4	-29.4	-69.2 ,	10.8
	1	-7.2	-50.6 ,	34.2	-30.0	-68.1 ,	10.6
	2	**-60.0**	**-104.4** ,	**-15.5**	22.3	-17.2 ,	61.0
	5	16.3	-46.7 ,	76.1	-0.6	-45.6 ,	46.1
	10	**95.3**	**18.7** ,	**169.5**	47.0	-2.7 ,	105.1
CD4:CD8 Ratio	0	**0.1**	**0.1** ,	**0.2**	0.0	-0.1 ,	0.2
	1	**0.1**	**0.1** ,	**0.2**	0.0	-0.1 ,	0.2
	2	**0.2**	**0.1** ,	**0.2**	0.1	0.0 ,	0.2
	5	0.1	0.0 ,	0.1	**-0.1**	**-0.3** ,	**0.0**
	10	0.1	0.0 ,	0.3	**-0.4**	**-0.5** ,	**-0.2**

Statistically significant results are in bold.

Causal mean differences were calculated using g-computation over 1,000 Monte Carlo simulations. Confidence intervals were calculated by summarizing over 1,000 simulations and reported the 2.5[th] and 97.5[th] percentile.
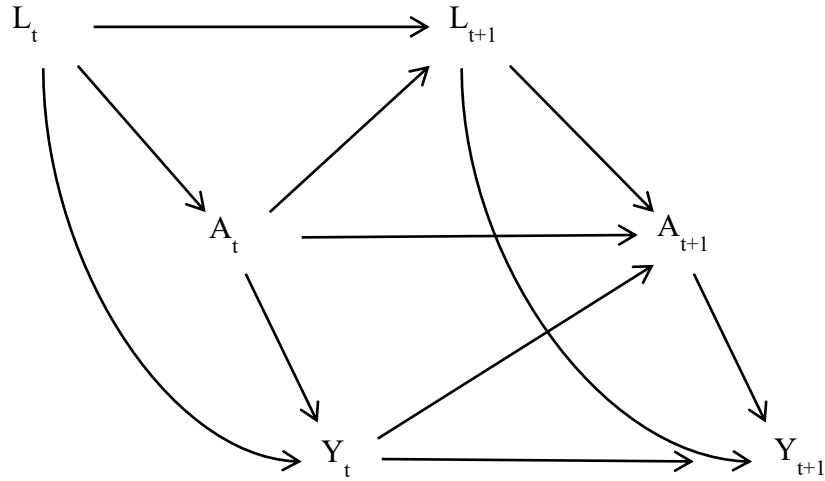
Figure 1. Directed acyclic graph of the study showing the relationships between exposure (A), outcome (Y) and confounding (L) variables over time (t), where t=1 to 20

Exposure (A) is the ED drug use and outcome (Y) is CD4 cell counts, CD8 cell counts, CD4 percentage of total lymphocytes, or CD4:CD8 ratio. Time-varying confounding variable (L) is the set of variables listed in Table 1. Time, where t=1 to 20, is the study visits for both HIV positive and negative groups of men in the MACS. In the causal intermediate path from A_t to Y_{t+1} through L_{t+1}, L_{t+1} to Y_{t+1} path is also on a biased path from A_{t+1} to Y_{t+1} through L_{t+1}. Other confounding variables of A on Y relationship at each time point was left out of the graph for convenience.
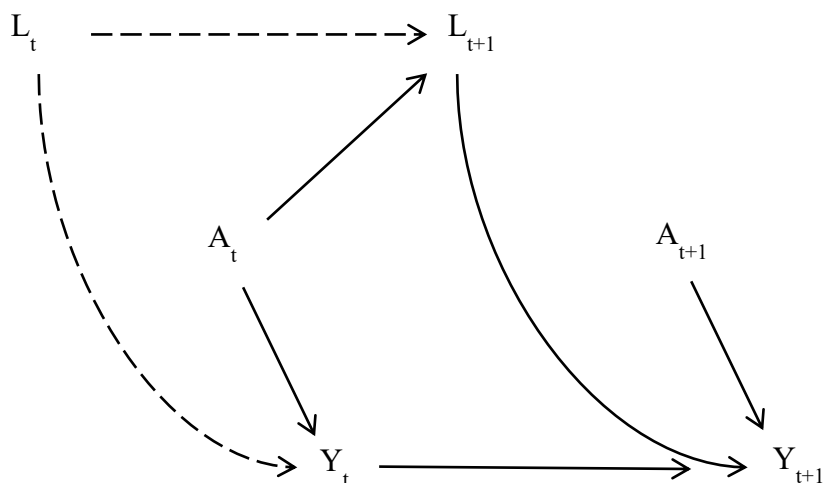
Figure 2. Directed acyclic graph of the study after intervention on the exposure (A) at each time point (t).

Time (t=1 to 20) is the study visits for both HIV positive and negative groups. Exposure (A) is the assigned ED drug use after intervention and outcome (Y) is the potential outcome for CD4 cell counts, CD4 percentage, CD8 cell counts, or CD4:CD8 ratio. After randomizing on A_t and A_{t+1} (assigning ED drugs), biasing paths from A_t and A_{t+1} are removed, while causal intermediate paths are preserved. Solid arrow lines represent the causal pathways of interest. Dashed arrow lines represent unbiased pathways after intervention on A. Pathways from other confounding variables to Y_t and Y_{t+1} were left out of the graph for convenience.
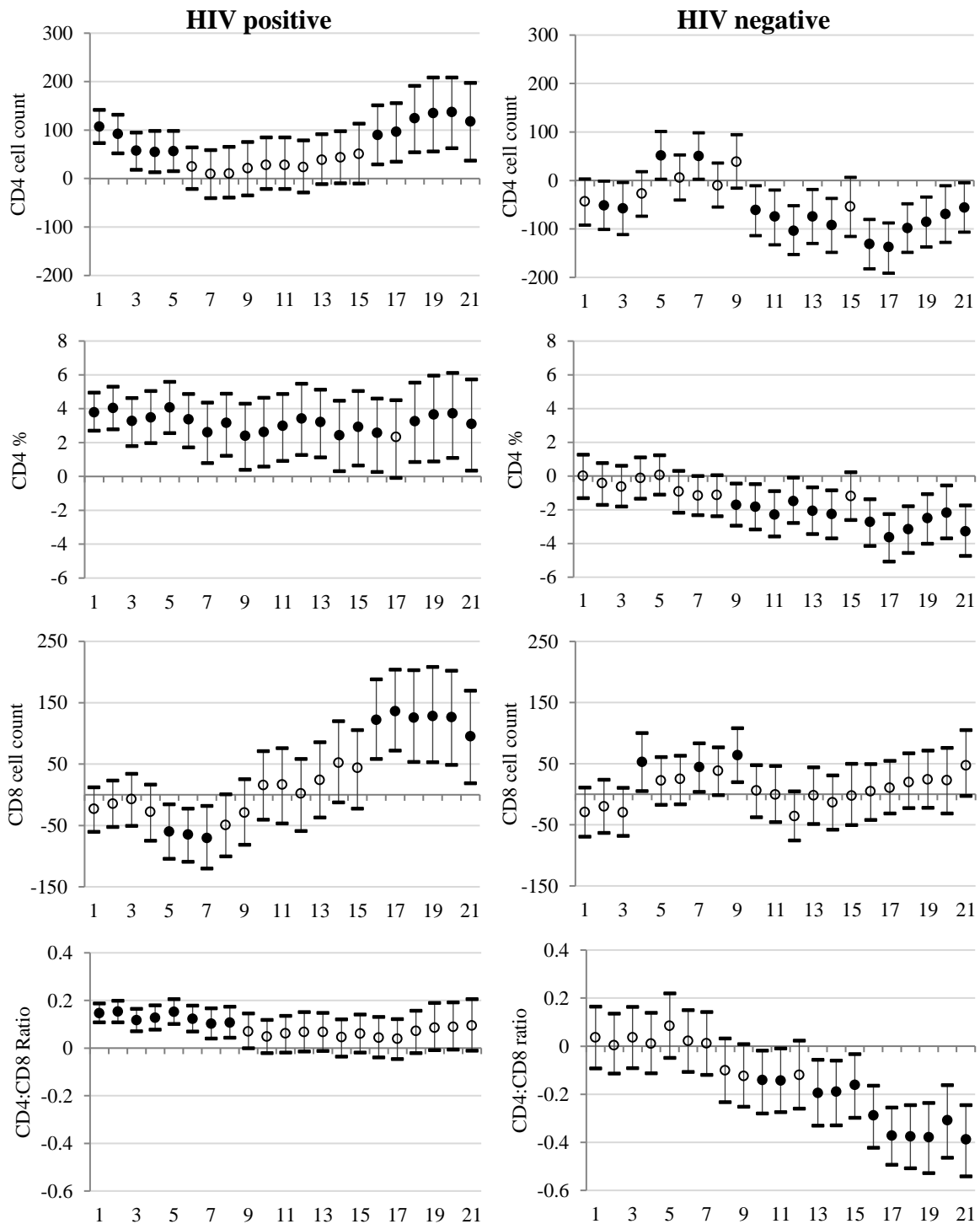
Figure 3. Causal mean differences and 95% confidence interval over all time points comparing ED drug users and non-users in HIV positive and negative subjects.

HIV positive group is shown in the left column and HIV negative group is on the right. Horizontal axis shows MACS study visits (1-21), where visit 1 is the baseline and visit 21 represents 10 years of follow-up. Filled circles represent statistically significant causal mean differences that were computed by marginal structural models using variables in Table 1.
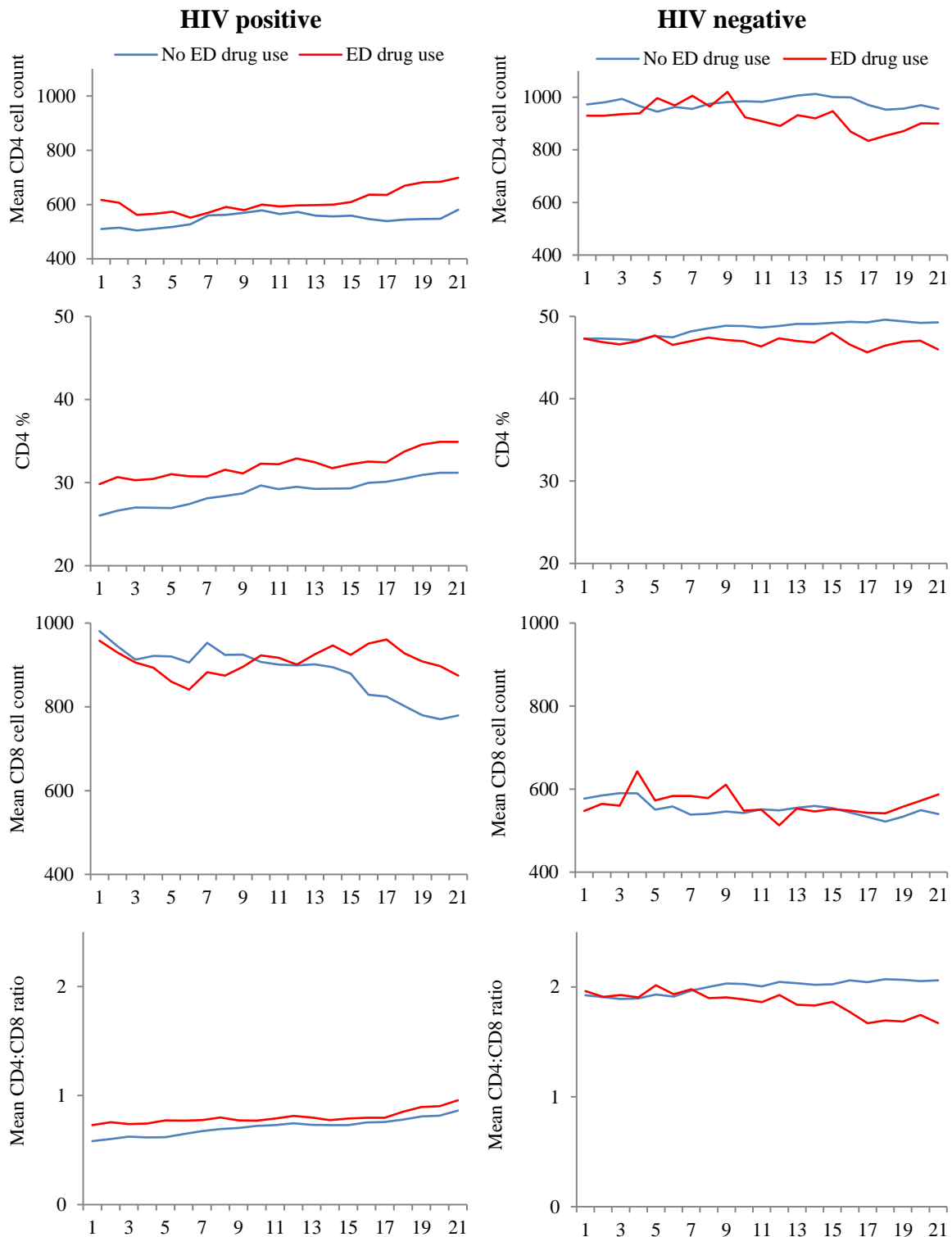
Figure 4. Mean CD4 cell counts, CD8 cell counts and CD4:CD8 ratio over time comparing ED drug users and non-users in HIV positive and negative subjects.

HIV positive group is shown in the left column and HIV negative group is on the right. Horizontal axis shows MACS study visits (1-21), where visit 1 is the baseline and visit 21 represents 10 years of follow-up. Red solid lines represent ED drug users and blue solid lines show non-users. Mean levels are computed by potential outcomes from marginal structural models using variables in Table 1.

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CHAPTER 5

Manuscript #3

Longitudinal effect of erectile dysfunction drugs on markers of immune activation and inflammation in men who have sex with men in the ARRA1 study of the MACS: Using G-computation

Abstract

Background: Erectile dysfunction is associated with HIV in men who have sex with men and drugs for erection problems are commonly used for both treatment and recreation. There is evidence of beneficial immunomodulatory effects of these drugs in animal studies; however, studies on the effect of erectile dysfunction drugs on the inflammation markers in men are limited.

Methods: The study participants were men who were in the ARRA1 sub-study of the Multicenter AIDS Cohort Study since 1998, when the erectile dysfunction drugs were available. We used g-computation for marginal structural models to assess the causal mean difference in markers of immune activation and inflammation over time. A total of 1,582 men, both HIV infected and uninfected, (age range: 19-70) were included in the analysis.

Results: Use of erectile dysfunction drugs over time in HIV positive men reduced levels of pro-inflammatory markers with causal mean differences in IL-6 of -1.98 (95% CI = -2.22 – -1.75) and TNF-α of -2.31 (-2.48 – -2.14) after 1 year of continuous use. Anti-inflammatory markers, such as IL-10 were higher with erectile dysfunction drug use after the first year with a causal mean difference of 1.36 (0.86 – 1.88). HIV negative men also showed similar significant results with erectile dysfunction drug use over time.

Conclusion: Erectile dysfunction drugs showed favorable immunomodulatory effects on the immune biomarkers in both HIV positive and negative men. Major pro-inflammatory markers were lower and anti-inflammatory markers were higher over time.

5.1. Background

Erectile dysfunction (ED) is common among men with HIV (*Romero-Velez, 2014*), and ED drugs are commonly used among men having sex with men (MSM) for the treatment of ED, especially in older men (*Fisher et al., 2006*), but also as a recreational drug without medical indication (*Harte and Meston, 2011; Kim et al., 2002*). Recreational uses of ED drugs were associated with other drug uses, such as marijuana and cocaine, as well as risky sexual activities (*Fisher et al., 2006; Purcell et al., 2005*). ED drugs, such as sildenafil, were first tested for the treatment of pulmonary arterial hypertension, however, as a selective and potent inhibitor of phosphodiesterase type 5 (PDE-5), which increases cyclic guanosine monophosphate (cGMP) levels, they were found to be more effective in the treatment of ED (*Barnett, 2006*). Furthermore, the inhibition of cGMP degradation in to GMP acts to regulate the levels and activities of immune cells and immune biomarkers (*Kniotek and Boguska, 2017*).

The effect of ED drugs on the human immune system is unclear. There are only a few studies that have examined the effect of these drugs on the immune markers in humans. In animal studies, ED drugs, mainly sildenafil, showed immunomodulatory effects in healthy mice (*Karakhanova et al., 2013; Szczypka and Obminska-Mrukowicz, 2010*) and in another study resulted in prolonged survival of tumor-bearing mice (*Serafini et al., 2006*). In a study that reported the effect of sildenafil on the human immune cells in vitro, positive effects on the healthy human lymphocytes were found (*Pifarre et al, 2014*).

HIV infection is characterized by several immune responses to an infection with the virus and the general immune activation by cytokines reacts to the viral replication inside the host (*Sauce et al, 2013*). So, HIV is associated with an increased expression of pro-inflammatory cytokines, such as TNF-alpha and IL-6, which are then associated with an up-regulation of

HIV replication (*Nixon and Landay, 2011*). In addition, immune-regulatory cytokines such as IL-2 and IL-12 are disrupted and lost, which then are not able to modulate an effective cell-mediated immune response via the T cells (*De Paoli, 2001*). Hence, circulating biomarkers of immune activation and inflammation can be measured and used in studies to examine the immune function and its responses.

There is some evidence of the potential immunomodulatory effects of ED drugs in the human immune system. However, the effect of ED drugs on the immune system in the MSM population has not been documented. Therefore, this study aimed to examine the effect of ED drugs on immune biomarkers over time in MSMs. Based on existing literature, it was hypothesized that ED drug users would have lower pro-inflammatory biomarkers and high anti-inflammatory biomarkers, which would suggest an improvement in the immune system over time compared to non-ED drug users.

5.2 Methods

5.2.1 Study Population

The participants were from the ARRA1 sub-study of the Multicenter AIDS Cohort Study (MACS), an ongoing prospective cohort study on MSMs from 4 study sites in the United States (Baltimore, Chicago, Pittsburgh, Los Angeles) (*Detels et al., 1992*). The purpose of the ARRA1 study was to examine the level of biomarkers among all HIV seroconverters and HAART initiators in the MACS during the course of the infection. This study has approximately 14,000 serum specimens that were tested from the MACS and includes serum samples from MACS person-visits that were chosen for inclusion in the study if the men had known HIV seroconversion dates and had annual lab visits from immediately before and after HAART initiation for all HAART users. Samples from HIV-uninfected men who were of similar age and race from 1984-2009 were also included.

For the purpose of this study, data from a total of 1,582 HIV positive and negative men were used in the analyses (age: 19-70 years). Subjects with only one visit in the study were excluded. In addition, study visits for treatment purposes were also omitted from the analysis. Subjects who were in the study from 1998 were included in the study since ED drugs were approved by the FDA in the market in 1998.

5.2.2 Measures

The exposure variable was ED drug use, which was self-reported use of ED drugs since the last visit. In addition to known ED drugs such as sildenafil, tadalafil and vardenafil, self-reported use of drugs that were not prescribed for ED was also included. Thus, the ED drug use included both prescribed and recreational use.

Covariates included in the study were demographic characteristics such as age, which was computed using date of birth and date of visit, race (white vs. other), education level (college degree vs. less than college) and study center (four study sites). Smoking status (smoker vs. non-smoker) and alcohol consumption (binge/heavy vs. moderate/light vs. non-drinker) were self-reported and obesity (obese/overweight vs. normal) was defined by BMI cut-offs of greater than 25. Other confounding variables included substance use (marijuana, poppers, stimulants, testosterone), medication (HAART, depression medication), and health-related factors such as HCV infection, and other co-morbidities (stroke, congestive heart problems, prostate cancer/surgery, bladder cancer/surgery) using ICD-9 codes. Sexual behavior variables included the number of men with whom the subjects engaged in unprotective insertive anal intercourse since the last visit (1 or more vs. 0), and number of male and female sex partners (2 or more vs. 0/1). Insertive anal intercourse was used since these would be the men who would use ED drugs. All variables except age, BMI, study center and health-related variables were self-reported. Covariates were selected based on prior studies and evidence of

host-factor influences on biomarkers (*Wada et al., 2015*; *McKay et al., 2016*).

A total of 24 immune markers were used as outcome variables. These were classified into three categories: 1) Cytokines, 2) Chemokines, and 3) Soluble receptors. Two multiplex assay platforms were used to measure the serum markers in the ARRA1 study. Nine cytokine markers (IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, TNF-α, GM-CSF) used Meso Scale Discovery system (MSD, Gaithersburg, MD) with Ultra-sensitive Human Pro-inflammatory 9-Plex Kit and seven chemokine markers (CCL2, CCL4, CCL11, CCL13, CCL17, CXCL10, CXCL8) used the MSD Ultrasensitie Human Chemokine 7-Plex Kit to quantify the levels. The Luminex platform (Luminex, Austin, TX) was utilized to measure the six soluble receptors (sCD14, sCD27, sGP130, sIL-2Rα, sIL-6R, sTNF-R2) as well as one cytokine (BAFF). Inflammatory markers with measures below the lower limit of detection (LLD) were assigned a value equal to the midpoint between the LLD and zero. Viral load was used as a continuous variable. Viral load and the immune markers were log-transformed.

5.2.3 Statistical Analysis

Subjects in the study were divided into two groups according to their HIV status (positive/negative). Descriptive statistics were used to show the baseline characteristics of subjects. Baseline was defined as the first visit made by participants after 1998 when the first ED drugs were available on the market. Baseline for the positive group included the first visit of HIV positive subjects in the study as well as the subjects who seroconverted during the follow-up of the study.

The ARRA1 data, like the MACS, is a longitudinal data set that includes time-varying exposure, confounders and outcome. We used g-computation to estimate the causal effect of ED drugs on the immune markers over time (*Robins, 1986; Robins, 2000*) in a longitudinal setting shown in the directed acyclic graph (DAG) in Figure 1. By applying a marginal

structural model, it was possible to eliminate bias arising from adjusting for time-varying confounding effects while preserving the intermediate paths (*Hernán et al., 2000; Robins et al., 2000; Hernán et al., 2001*). Figure 2 shows the causal DAG after the intervention at each exposure. Here, confounding effects by the time-varying variables are eliminated while the intermediate pathways from previous exposure to the next outcome remain, so that we are able to compute an unbiased estimate of the causal relation between exposure and the outcome.

If we let A_t be exposure, Y_t be outcome, L_t is a set of covariates (confounding variables) where t represents time/visit (t=1, 2,…, 21), Y_a is the potential outcome of Y when A is set to a and *newA* is when we set A to be a. Firstly, we specified and fitted the models for the outcome of interest, $E(Y_t|A_t, L_t, Y_{t-1})$, and the models for time-varying confounders, L's: $P(L_t|A_{t-1}, L_{t-1})$, using the observed Y's, A's, and L's in PROC GENMOD and saved the regression coefficients. Next, we created two copies of the data, where one was given *newA*=1 (all subjects took ED drugs) and the other was given *newA*=0 (all subjects did not take ED drugs). Then, using Markov Chain Monte Carlo simulation with 1,000 repetitions, we created *newL*'s and new potential outcomes, $E(Y_a|newA, newL)$, in each time point using the saved parameters. Finally, we regressed Y_a on *newA* to estimate the causal mean difference using PROC GENMOD ($E(Y_{a=1}) - E(Y_{a=0})$). Confidence intervals were computed by summarizing the results over the repetitions using 2.5th and 97.5th percentile as the lower and upper confidence limits. This study included multiple A's, L's and Y's over time, so construction of new variables was repeated for each time point to account for these extra terms. Interactions between ED drug use and time-fixed variables, such as age, race and education were included in the model. Interactions terms between ED drugs and other confounding variables were tested but showed no statistical significance and were not included. Variables included in the

steps for g-computation are shown in Table 1.

Values from previous visits were used to replace missing values for the variables that were "used since last visit". Several variables that did not change their values from previous to current visits, such variables included smoking, alcohol consumption, recreational drugs, obesity, HCV infection and comorbidities. Intermittent missing data were not dependent on subject-specific characteristics and no adjustment was made in the analysis. Data were analyzed in SAS 9.4 (SAS Institute Inc., Cary, North Carolina).

5.3 Results

Data from 1,148 HIV positive men and 286 HIV negative men was available for analysis. Overall, the ARRA-1 study participants had an average of 4.8±2.9 visits by HIV positive men and 2.6±1.0 visits by HIV negative men (data not shown). Table 2 shows the descriptive baseline statistics of ED drug users and non-users among the subjects that were included in the analyses. At baseline, 14.7% of HIV negative men reported ED drug use compared to 8.2% of HIV positive men. The mean age among HIV positive was higher in ED drug users, 46.8±8.4 years compared to non-users with 43.3±7.8 years. ED drug users were also older among HIV negative men (ED drug user: 49.2±9.2 vs. non-user: 47.0±9.7 years). Most of the HIV positive and negative study subjects were white, non-smokers and non-drinkers of alcohol and had a normal BMI. The education level was similar in all groups. A higher proportion of subjects reported recreational drugs use among ED drug users in both HIV positive and negative groups. The majority of HIV positive men were on HAART at baseline (56.1% total; 57.1% non-users, 45.3% ED drug users). HCV infection was higher among ED drug users in both groups of men at baseline but was not significantly different. Both groups of men had a similar proportion of at least one pre-existing condition. ED drug users reported a higher proportion of having more than 2 sex partners, 42.0% of ED drug users in HIV

positive and 42.9% of HIV negative men were engaged in unprotected insertive anal intercourse at baseline. Table 3 shows the descriptive statistics for the immune markers as well as the viral load before log-transformation in both HIV groups.

Table 4 shows the causal mean differences (MD) comparing ED drug users and non-users for the log-transformed immune biomarkers among HIV positive men for 5 study visits including the baseline. The causal MDs for 5 inflammatory cytokines (BAFF, IL-6, IL-8-pro, IFN-γ, TNF-α) showed favorable results with ED drug use over time but IL-1β showed no significant difference and was higher after 1.5 years (Table 4a). After 6 months, pro-inflammatory cytokine IL-6 was an average of 0.70 lower in users than non-users (95% CI: -0.86–-0.54) and after 1 year from the first visit, mean IL-6 was lower by 1.98 (95% CI: -2.22–-1.75). Anti-inflammatory cytokines IL-2, IL-10 and IL-12p70 also showed positive results. These 3 cytokines were higher in ED drug users compared to non-users and continued to be higher over time. Mean IL-2 levels were greater in ED drug users with a causal MD of 1.62 (95% CI: 1.22–2.01) after 1 year and at the same time, IL-10 mean level was higher by 1.36 (95% CI: 0.86–1.88). Chemokines CCL2, CCL4, CCL11, CXCL8, CXCL10, CXCL13 were lower with ED drug use over time (Table 4b). Four soluble receptors were slightly higher in ED drug users but sIL-2Rα and sTNF-R2 showed no significant difference (Table 4c).

The causal MD's and confidence intervals for HIV negative men are in Tables 5a-c. Three pro-inflammatory cytokines (IL-1β, IL-6, GM-CSF) showed beneficial results (Table 5a). At the first follow-up visit, ED drug users had a lower mean IL-1β (MD: -1.05, 95% CI: -1.29–-0.82) and IL-6 (MD: -0.66, 95% CI: -0.84–-0.49). TNF-α was lower in users at baseline but was no longer statistically significant after the first follow-up visit. Other pro-inflammatory markers such as BAFF, IL-8-pro, and IFN-γ did not exhibit favorable results as they were

higher in ED drug users than non-users. Like the HIV positive group, anti-inflammatory markers IL-2 and IL-10 were estimated to be higher in users after the first follow-up visit (IL-10 MD: 0.70, 95% CI: 0.47–0.93). Table 5b shows 4 chemokines that were greater in ED drug users (CCL2, CCL11, CXCL8, CXCL10) while CCL13 and CCL17 levels were lower. With soluble receptors, 4 out of 6 markers (sIL-2Rα, sTNF-R2, sCD4, sCD27) were higher in ED drug users (Table 5c).

Figure 3 shows the causal MD's and 95% CI's for all 24 immune markers over time for HIV positive (3a) and negative (3b) groups. The figures re-iterate the results from Tables 4 and 5, and clearly illustrate the average differences of all markers with ED drug use for each time point. The mean levels of immune markers over time in ED drug users and non-user among HIV positive and negative subjects are shown in Figures 4a and 4b, respectively.

5.4 Discussion

The longitudinal data from this study demonstrated immunomodulatory effects of ED drugs on markers of immune activation and inflammation in MSMs. In HIV positive subjects, 5 pro-inflammatory markers (BAFF, IL-6, IL-8pro, IFN-γ, TNF-α) were lower in ED drug users compared to non-users, while anti-inflammatory markers were higher (IL-2, IL-10, IL-12p70). Most of the chemokines and some soluble receptors were also lower in ED drug users than non-users in the same group of men. Although the same results were not observed in HIV negative subjects several important markers changed in the expected direction. IL-1β, IL-6 and TNF-α were both lower whereas IL-10 was higher in ED drug users. The 2 chemokines that were lower in the HIV negative group were CCL13 and CCL17, but most chemokines and soluble receptors were elevated in ED drug users.

The results from this study were similar to previous findings on the effect of ED drugs on immune markers. IL-6 is an important pro-inflammatory cytokine and is associated with

untreated HIV infection, disease progression and mortality (*Nixon and Landay, 2010*; *Deeks, 2011; Siewe and Landay, 2018*). In a study of healthy mice, sildenafil reduced the level of serum IL-6 (*Karakhanova et al., 2013*), and in patients with diabetes, chronic use of PDE5 inhibitors demonstrated a decrease in IL-6 by improving NO production (*Aversa et al., 2008; Santi et al., 2015*). In our study, levels of IL-6 were also declining over time in both HIV positive and negative MSMs.

Other important inflammatory markers, such as IL-1β, TNF-α and IFN-γ were also reduced in animal studies with ED drugs. In sildenafil-treated mice, levels of TNF-α and IFN-γ were reduced (*Pifarre et al., 2014*) while another study demonstrated the same results with IL-1β also decreased (*Nunes et al., 2012; Nunes et al., 2015*). Moreover, sildenafil-treated rats with lung fibrosis showed an attenuation of the levels of IL-1β and TNF-α (*Yildirim et al., 2010*). The LPS-induced (lipopolysaccharide) expression of these cytokines by microglial cells were also reduced when sildenafil was administered (*Zhao et al., 2011*). The estimated TNF-α level declined over time among ED drug users in the current study, however, IFN-γ in HIV positive subjects and IL-1β in HIV negative subjects were reduced. There was no difference in IL-1β levels in HIV positive men between ED drug users and non-users until later, which was also seen in other studies (*Karakhanova et al., 2013; Szczypka and Obmiska-Mrukowicz, 2010)*.

In accordance with our results, anti-inflammatory markers such as IL-2 and IL-10 were increased in several studies (*Karakhanova et al., 2013; Nunes et al., 2015*). However, there are other studies that have shown opposite effects or no changes in the levels of IL-2 or IL-10 (*Szczypka et al., 2012; Pifarre et al., 2014, Nunes et al., 2012; Nunes et al,. 2015*). The levels of these two cytokines were greater in ED drug users than non-users in both HIV groups. IL-12p70, similar to IL-2, increases the immune T-cell activation, and was also higher in HIV positive men, but not in the negative group. The higher levels of anti-inflammatory cytokines

in both groups of men may suggest increased immune competence from ED drug use.

Despite the treatment with ARTs in HIV infected individuals, levels of sCD14 and sCD27 persist at a high level (*Deeks, 2011; Siewe and Landay, 2018; Wada et al., 2015*). Elevated levels of these soluble receptors did not change with ED drug use in the HIV positive group and was also higher in the negative group. CXCL10 and sTNF-R2 was also observed to be high among HIV-suppressed subjects in another ARRA1 study by Wada et al. (2015) but these markers declined over time with ED drug use in this study.

The results from this study must be carefully interpreted since they deal with potential outcomes in marginal structural models. These provide unbiased estimates of the causal mean difference in markers of immune activation and inflammation between ED drug users and non-users. The causal MD of the inflammatory marker, IL-6, after the first follow-up visit (-0.70, 95% CI: -0.86–-0.54) means that men who would have self-reported taking the ED drugs both at baseline and the first follow-up visit, had an average difference of 0.70 compared to men who would have reported not taking the ED drugs at baseline and the first follow-up visit.

It is difficult to directly compare our results with other studies due to the implementation of marginal structural models in our analysis. Like all causal inference methods, g-computation requires assumptions about the model for valid analyses. Key causal assumptions include conditional exchangeability (no uncontrolled confounding), consistency and positivity (*Hernan and Robins, 2019*). Specific to this study, there were three assumptions. First, the assumption was that the measured covariates in L_t were sufficient to adjust for confounding. This implied that for each semiannual visit t, there was accurate data available in L_t on all time-dependent covariates that were independent predictors of the biomarkers and also, independent predictors of using ED drugs in that visit. Second, an assumption on the accurate

information on ED drug use and the levels of biomarkers was made. If we assume that the effect of ED drugs, regardless of the source, is the same for everyone in the study, then we can assume consistency. Lastly, the marginal structural model for the effect of ED drugs on immune markers, within levels of covariates *L*, was assumed to be correctly specified (*Hernán et al., 2000*).

There are several limitations to this study. The present study did not look at the frequency or dosage of ED drugs as they were not available. Separate analyses for different types of ED drugs were also not examined. It was not possible to distinguish between the drugs as there were insufficient data on types of drug used during each visit. Prescription data for drugs was not available for subject, and so the ED drug use could not be linked to confirm their self-reported responses. Social demographic information, as well as recreational drugs and sexual activities were from self-reported data. Even though the use of ACASI helped participants respond to sensitive questions more accurately, there could still have been misreporting of information. Partner status was unknown and pre-exposure prophylaxis (PrEP) was not included in the analysis as it was made available by the FDA in 2012, and was not available for the majority of the study period. Biomarkers could have degraded in storage of the specimens studied. However, this would be a non-differential measurement error which would bias the estimates toward the null.

To our knowledge, this is the first study that examined the use of ED drugs over time in humans, and in MSMs. Most of the prior evidence was from animal experiments, and there was a lack of studies looking at the longitudinal data of ED drugs in humans. Other strengths of this study included the use of prospectively collected data and specimens from a well-defined cohort of MSMs in the U.S. This data allowed us to examine the effect of long-term use of ED drugs on the immune markers, which included important subpopulations and

socially sensitive data collected through a standardized method for data collection. Centralized laboratories also allowed minimization in variability of biomarkers. Furthermore, inclusion of HIV negative MSMs in the study allowed us to analyze both groups of subjects. The marginal structural model method used in the analyses had several advantages. G-computation was able to deal with time-varying exposure and confounding variables while producing unbiased and efficient estimates with small standard errors (*Daniel et al., 2013; Wang et al., 2017*).

5.5 Implications

The anti-inflammatory effects of ED drugs in MSMs may have some clinical implications. Residual inflammation after successful treatment with anti-retroviral therapy in men with HIV has been a problem because of its association with disease progression and persistence of HIV (*Klatt et al., 2013; Massanella, et al., 2017*). Participants in the MACS also showed high levels of inflammation remained after HIV suppression, although they were restored after one year of viral suppression with HAART (Wada et al., 2015). If PDE5 inhibitors convincingly demonstrate enhanced immunity, suggested by elevated levels of IL-10 and lower IL-6, as well as other immunosuppressive effects, the use of ED drugs may not only be limited to ED treatment, but to other clinical settings to dampen the level of inflammation. There have been reports in the past on the unexpected treatment of treatment of B-cell chronic lymphocytic leukemia (B-CLL) in one patient with sildenafil (*Sarfati et al., 2003; Peak et al., 2016*) and improvements in patients with autoimmune diseases as well as neurodegenerative disorders when sildenafil was administered (*Kniotek and Boguska, 2017*). Furthermore, PDE5 inhibitors have shown to improve chemotherapy efficacy in different cancer cells (*Black et al., 2008; Das et al., 2010; Di et al., 2010*). Thus, there may be a place for ED drugs as additional treatment in various clinical settings.

5.6 Conclusion

The data from this study suggested that MSMs who took ED drugs over time had higher levels of anti-inflammatory cytokines and lower levels of pro-inflammatory cytokines and chemokines when compared with men who would have reported not using ED drugs. HIV negative MSMs also showed similar results although some markers moved in opposite directions or were no different between users and non-users. We believe this study has contributed to the growing body of evidence showing the immunomodulatory effects of ED drugs and additionally demonstrate the long-term effects of these drugs on immune markers in MSMs. Further studies are needed to provide a greater understanding on the effects of ED drugs in experimental and clinical settings.

Table 1. Summary of variables used in the analysis.

	Outcome Variables	Outcome model (Y)	Time-varying confounder model (L)
Cytokines	BAFF	ED drug	ED drug[*]
	IL-1β	Age	Age[*]
	IL-2	Race	Race[*]
	IL-6	Education level	Education level[*]
	IL-8pro	Smoking	Smoking[*]
	IL-10	Alcohol consumption	Alcohol consumption[*]
	IL-12p70	Obesity	Obesity[*]
	IFN-γ	Marijuana	Marijuana[*]
	TNF-α	Poppers	Poppers[*]
	GM-CSF	Stimulants	Stimulants[*]
Chemokines	Eotaxin/CCL11	Testosterone[†]	Testosterone[*†]
	MCP-1/CCL2	Depression medication	Depression medication[*]
	MCP-4/CCL13	HAART[†]	HAART[*†]
	MIP-1β /CCL4	HCV infection	HCV infection[*]
	TARC/CCL17	Co-morbidities	Co-morbidities[*]
	BLC/BCA1/CXCL13	UIAI	UIAI[*]
	IL-8/CXCL8	Number of sex partners	Number of sex partners[*]
	IP-10/CXCL10	Study center	
Soluble receptors	sIL-2Rα	Viral load[†]	
	sIL-6R	Previous outcome[*]	
	sTNF-R2		
	sCD14		
	sCD27		
	sGP130/CD130		

HAART: Highly Active Antiretroviral Therapy. HCV: Hepatitis C Virus. UIAI: Unprotected Insertive Anal Intercourse
*Lagged variable (previous visit)
†Variable not included in the HIV negative group analyses

Table 2. Descriptive baseline characteristics among HIV positive and negative men in the ARRA1 study who reported ED drug use since last visit compared to those who did not report ED drug use since last visit.

	Erectile Dysfunction Drug Use					Erectile Dysfunction Drug Use			
HIV Positive	No (n=1,053)		Yes (n=95)		HIV Negative	No (n=244)		Yes (n=42)	
	N	(%)	N	(%)		N	(%)	N	(%)
Age[*][§]	43.3	± 7.8	46.8	± 8.3	Age[*]	47.0	± 9.7	49.2	± 9.2
Non-white	449	(42.6)	35	(36.8)	Non-white	119	(48.8)	16	(38.1)
Education (College or higher)	527	(50.1)	48	(50.5)	Education (College or higher)	124	(50.8)	24	(57.1)
Smoker	388	(36.9)	38	(40.0)	Smoker	105	(43.0)	13	(31.0)
Alcohol consumption					Alcohol consumption	73	(29.9)	13	(31.0)
Binge/Heavy	86	(8.2)	8	(8.4)					
Low/Moderate	187	(17.8)	24	(25.3)					
Obesity/Overweight (BMI>25)	112	(10.6)	7	(7.4)	Obesity/Overweight (BMI>25)	50	(20.5)	9	(21.4)
Marijuana	394	(37.4)	42	(44.2)	Marijuana	65	(26.6)	12	(28.6)
Poppers[§]	272	(25.8)	42	(44.2)	Poppers	50	(20.5)	14	(33.3)
Stimulants[†][§]	211	(20.0)	32	(33.7)	Stimulants[†]	56	(23.0)	10	(23.8)
Depression medication[§]	250	(23.7)	34	(35.8)	Depression medication	40	(16.4)	10	(23.8)
Testosterone[§]	47	(4.5)	11	(11.6)					
HAART[§]	601	(57.1)	43	(45.3)					
HCV infection	104	(9.9)	13	(13.7)	HCV infection	50	(20.5)	9	(21.4)
Pre-existing conditions[‡]	60	(5.7)	7	(7.4)	Pre-existing conditions[‡]	14	(5.7)	2	(4.8)
Sex partners (≥2 partners)[§]	425	(40.4)	60	(63.2)	Sex partners (≥2 partners)[§]	107	(43.9)	31	(73.8)
UIAI (≥1 partner)[§]	213	(17.3)	34	(42.0)	UIAI (≥1 partner)[§]	66	(27.1)	18	(42.9)
Study site (Center)					Study site (Center)				
Baltimore	257	(24.4)	26	(27.4)	Baltimore	53	(21.7)	12	(28.6)
Chicago	262	(24.9)	26	(27.4)	Chicago	55	(22.5)	12	(28.6)
Pittsburgh	216	(20.5)	15	(15.8)	Pittsburgh	87	(35.7)	13	(31.0)
Los Angeles	318	(30.2)	28	(29.5)	Los Angeles	49	(20.1)	5	(11.9)

* All continuous variables are expressed as: mean ± s.d.

† Include cocaine, ecstasy, methamphetamine, and uppers.

‡ Include stroke, coronary heart failure, prostate surgery/cancer, bladder surgery/cancer.

§ $p<0.05$.

BMI: Body Mass Index. HAART: Highly Active Antiretroviral Therapy. HCV: Hepatitis C Virus. UIAI: Unprotected insertive anal intercourse.

Table 3. Descriptive baseline characteristics of immune biomarkers among HIV positive and negative men in the ARRA1 study who reported ED drug use compared to those who did not report ED drug use since last visit.

	Erectile Dysfunction Drug Use					Erectile Dysfunction Drug Use			
HIV Positive	No (n=1,053)		Yes (n=95)		HIV Negative	No (n=244)		Yes (n=42)	
	Median	IQR	Median	IQR		Median	IQR	Median	IQR
Viral load	176	40 - 13600	534	40 - 28495					
BAFF	2299.8	1887.7 - 2968.8	2225.3	1947.9 - 2608.4	BAFF	1968.1	1723.4 - 2263.6	1995.6	1629.6 - 2216.9
IL-1β	0.26	0.16 - 0.50	0.28	0.14 - 0.51	IL-1β	0.28	0.28 - 0.49	0.25	0.14 - 0.57
IL-2	0.60	0.30 - 0.99	0.57	0.33 - 1.07	IL-2	0.46	0.30 - 0.83	0.51	0.30 - 1.06
IL-6	0.98	0.68 - 1.57	1.01	0.62 - 1.60	IL-6	0.95	0.63 - 1.49	0.79	0.61 - 1.19
IL-8pro	14.4	9.91 - 57.9	14.4	11.1 -24.2	IL-8pro	14.9	10.7 - 20.1	12.5	10.0 - 19.7
IL-10	3.08	1.96 - 5.09	3.29	2.11 - 5.01	IL-10	3.20	1.93 - 6.67	2.88	1.67 - 5.79
IL-12p70	1.59	0.88 - 3.09	1.75	1.02 - 2.75	IL-12p70	2.25	1.09 - 5.78	1.84	1.04 - 5.27
IFN-γ	1.04	0.64 - 1.90	1.06	0.66 - 1.76	IFN-γ	0.94	0.53 - 1.62	0.86	0.56 - 1.26
TNF-α	10.4	7.9 - 14.5	10.3	8.03 - 16.21	TNF-α	8.77	6.88 - 11.23	8.17	6.88 - 11.3
GM-CSF	0.54	0.24 - 1.02	0.47	0.24 - 1.24	GM-CSF	0.62	0.33 - 1.27	0.66	0.26 - 1.10
Eotaxin	1633.3	1182.6 - 2251.0	1662.0	1277.4 - 2246.7	Eotaxin	1592.2	1141.1 - 2322.8	1837.1	1276.9 - 2408.7
MCP-1	568.8	412.1 - 760.4	564.6	402.3 - 709.1	MCP-1	492.9	365.9 - 662.6	565.4	493.2 - 700.0
MCP-4	746.0	549.1 - 1003.5	752	555.7 - 1016.8	MCP-4	819.8	596.7 - 1048.3	841.3	613.6 - 1056.1
MIP-1β	123.1	83.1 - 177.9	112.2	73.2 - 157.8	MIP-1β	155.7	94.7 - 200.9	137.8	84.8 - 193.8
TARC	520.4	328.7 - 824.5	518.9	323.6 - 861.2	TARC	542.0	385.4 - 890.0	517.9	363.6 - 753.8
BLC/BCA1	309.4	251.3 - 387.1	316.6	267.0 - 379.1	BLC/BCA1	287.1	242.8 - 347.0	293.4	244.7 - 341.2
IL-8	13.4	9.1 - 23.7	14.8	10.3 - 21.2	IL-8	13.1	9.3 -19.3	13.0	9.6 - 18.4
IP-10	266.9	167.8 - 472.2	306.7	171.5 - 549.3	IP-10	143.9	96.8 - 233.9	173.4	99.8 - 289.9
sIL-2Rα	1710.7	1294.2 - 2304.6	1876.5	1385.4 - 2408.6	sIL-2Rα	1425.1	1159.7 - 1732.7	1345.8	1136.9 - 1693.6
sIL-6R	51039.6	41771.5 - 61401.2	53468.8	42578.4 - 64448.2	sIL-6R	49510.6	40485.5 - 60574.2	48771.5	41307.2 - 60612.5
sTNF-R2	3125.4	2409.2 - 4263.3	3075.8	2357.5 - 4583.1	sTNF-R2	2423.2	1994.6 - 2983.9	2188.7	1890.4 - 2847.2
sCD14	2576903	2147213 - 3099300	2624636	2180124 - 3027177	sCD14	2144964	1837518 - 2490452	2086881	1841169 - 2405069
sCD27	12850.6	9641.0 - 17487.2	13467.5	10753.2 - 17804.7	sCD27	9443.9	7654.1 - 11785.7	9084	7588.1 - 11311.7
sGP130	269732	237185 - 316705	276196	238718 - 323014	sGP130	261487	231471 - 300588	247664	224766 - 280060

Table 4. Causal mean differences (MD) and 95% confidence intervals (CI) for immune biomarkers in ED drug users and non-users among HIV positive men across 5 study visits.

a. Causal MD and 95% CI's for cytokines over 5 time points.

Marker	Year	MD	95% CI			Marker	Year	MD	95% CI		
BAFF	0	**-0.08**	**-0.11**	,	**-0.05**	IL-10	0	0.07	-0.01	,	0.16
	0.5	**-0.10**	**-0.16**	,	**-0.03**		0.5	0.02	-0.14	,	0.19
	1	**-0.38**	**-0.50**	,	**-0.25**		1	**1.36**	**0.86**	,	**1.88**
	1.5	**-0.28**	**-0.41**	,	**-0.15**		1.5	**0.80**	**0.30**	,	**1.35**
	2	0.26	-0.32	,	0.86		2	0.72	-0.10	,	1.50
IL-1β	0	0.03	-0.05	,	0.11	IL-12p70	0	**0.20**	**0.09**	,	**0.31**
	0.5	-0.11	-0.30	,	0.09		0.5	-0.19	-0.41	,	0.02
	1	0.09	-0.26	,	0.44		1	**3.18**	**2.47**	,	**3.93**
	1.5	**0.60**	**0.09**	,	**1.09**		1.5	**2.77**	**1.89**	,	**3.58**
	2	**1.67**	**0.41**	,	**2.99**		2	**2.27**	**0.38**	,	**4.21**
IL-2	0	**-0.07**	**-0.14**	,	**0.00**	IFN-γ	0	-0.06	-0.13	,	0.01
	0.5	-0.14	-0.32	,	0.03		0.5	**-0.21**	**-0.36**	,	**-0.07**
	1	**1.62**	**1.22**	,	**2.01**		1	**-0.70**	**-1.00**	,	**-0.42**
	1.5	**0.90**	**0.49**	,	**1.29**		1.5	**-0.35**	**-0.71**	,	**-0.02**
	2	0.96	-0.02	,	1.97		2	0.12	-0.99	,	1.19
IL-6	0	-0.06	-0.13	,	0.01	TNF-α	0	-0.02	-0.06	,	0.03
	0.5	**-0.70**	**-0.86**	,	**-0.54**		0.5	**-0.12**	**-0.21**	,	**-0.03**
	1	**-1.98**	**-2.22**	,	**-1.75**		1	**-2.31**	**-2.48**	,	**-2.14**
	1.5	**-1.15**	**-1.47**	,	**-0.83**		1.5	**-0.58**	**-0.73**	,	**-0.43**
	2	-0.91	-2.34	,	0.59		2	**-0.49**	**-0.77**	,	**-0.20**
IL-8pro	0	0.02	-0.06	,	0.10	GM-CSF	0	-0.06	-0.15	,	0.04
	0.5	-0.19	-0.39	,	0.00		0.5	0.00	-0.19	,	0.19
	1	**-1.20**	**-1.65**	,	**-0.75**		1	**0.63**	**0.16**	,	**1.07**
	1.5	**-0.83**	**-1.33**	,	**-0.38**		1.5	0.05	-0.58	,	0.64
	2	**-1.51**	**-2.40**	,	**-0.65**		2	0.21	-2.14	,	2.53

Significant results are in bold.

b. Causal MD and 95% CI's for chemokines over 5 time points.

Marker	Year	MD	95% CI			Marker	Year	MD	95% CI		
Eotaxin	0	-0.02	-0.06	,	0.02	TARC	0	0.05	-0.01	,	0.11
(CCL11)	0.5	-0.03	-0.12	,	0.05	(CCL17)	0.5	-0.03	-0.17	,	0.12
	1	**-0.42**	**-0.63**	**,**	**-0.20**		1	-0.11	-0.39	,	0.15
	1.5	**-0.27**	**-0.53**	**,**	**-0.03**		1.5	0.27	-0.14	,	0.67
	2	0.28	-0.14	,	0.73		2	-0.33	-1.04	,	0.38
MCP-1	0	**-0.10**	**-0.14**	**,**	**-0.07**	BLC/BCA1	0	0.00	-0.04	,	0.05
(CCL2)	0.5	-0.03	-0.11	,	0.05	(CXCL13)	0.5	-0.04	-0.14	,	0.06
	1	**-0.98**	**-1.19**	**,**	**-0.76**		1	**-0.34**	**-0.49**	**,**	**-0.18**
	1.5	**-0.72**	**-0.95**	**,**	**-0.50**		1.5	-0.12	-0.26	,	0.02
	2	-0.03	-0.79	,	0.67		2	**-0.47**	**-0.79**	**,**	**-0.17**
MCP-4	0	0.03	-0.01	,	0.07	IL-8	0	-0.01	-0.09	,	0.07
(CCL13)	0.5	0.06	-0.02	,	0.14	(CXCL8)	0.5	**-0.22**	**-0.42**	**,**	**-0.03**
	1	0.14	-0.09	,	0.38		1	**-1.23**	**-1.70**	**,**	**-0.74**
	1.5	0.23	-0.03	,	0.48		1.5	**-0.93**	**-1.46**	**,**	**-0.38**
	2	0.30	-0.22	,	0.81		2	**-1.05**	**-1.99**	**,**	**-0.09**
MIP-1β	0	**-0.09**	**-0.15**	**,**	**-0.03**	IP-10	0	**0.07**	**0.01**	**,**	**0.13**
(CCL4)	0.5	-0.08	-0.21	,	0.05	(CXCL10)	0.5	-0.05	-0.18	,	0.08
	1	**-2.51**	**-2.79**	**,**	**-2.23**		1	-0.10	-0.33	,	0.13
	1.5	**-1.38**	**-1.66**	**,**	**-1.09**		1.5	**-0.39**	**-0.70**	**,**	**-0.06**
	2	0.04	-0.45	,	0.51		2	-0.45	-1.40	,	0.52

Significant results are in bold.

c. Causal MD and 95% CI's for soluble receptors over 5 time points.

Marker	Year	MD	95% CI			Marker	Year	MD	95% CI		
sIL-2Rα	0	0.02	-0.01	,	0.05	sCD14	0	**0.05**	**0.02**	**,**	**0.08**
	0.5	-0.02	-0.10	,	0.05		0.5	**-0.11**	**-0.16**	**,**	**-0.05**
	1	**-0.33**	**-0.50**	**,**	**-0.17**		1	**0.79**	**0.72**	**,**	**0.87**
	1.5	**-0.19**	**-0.35**	**,**	**-0.04**		1.5	**0.32**	**0.21**	**,**	**0.44**
	2	-0.33	-1.03	,	0.38		2	0.84	-0.39	,	2.08
sIL-6R	0	-0.15	-0.23	,	-0.06	sCD27	0	0.02	-0.02	,	0.05
	0.5	-0.86	-1.11	,	-0.61		0.5	-0.02	-0.09	,	0.05
	1	0.25	-0.24	,	0.74		1	**0.27**	**0.11**	**,**	**0.43**
	1.5	0.34	-0.29	,	1.00		1.5	0.09	-0.07	,	0.24
	2	-0.06	-0.70	,	0.60		2	-0.14	-0.52	,	0.24
sTNF-R2	0	-0.01	-0.04	,	0.03	sGP130	0	**0.05**	**0.03**	**,**	**0.07**
	0.5	-0.02	-0.09	,	0.05	(CD130)	0.5	**0.21**	**0.16**	**,**	**0.26**
	1	-0.03	-0.15	,	0.10		1	**0.52**	**0.42**	**,**	**0.63**
	1.5	**-0.15**	**-0.30**	**,**	**-0.02**		1.5	**0.33**	**0.24**	**,**	**0.41**
	2	-0.22	-0.85	,	0.42		2	**-2.05**	**-3.08**	**,**	**-1.04**

Significant results are in bold.

Table 5. Causal mean differences (MD) and 95% confidence intervals (CI) for immune biomarkers in ED drug users and non-users among HIV negative men across 2 study visits.

a. Causal MD and 95% CI's for cytokines over 2 time points.

Marker	Year	MD	95% CI		Marker	Year	MD	95% CI	
BAFF	0	-0.02	-0.06 ,	0.01	IL-10	0	-0.21	-0.42 ,	0.02
	0.5	**0.08**	**0.04 ,**	**0.11**		0.5	**0.70**	**0.47 ,**	**0.93**
IL-1β	0	-0.09	-0.27 ,	0.08	IL-12p70	0	-0.26	-0.51 ,	0.01
	0.5	**-1.05**	**-1.29 ,**	**-0.82**		0.5	**-0.40**	**-0.65 ,**	**-0.14**
IL-2	0	0.06	-0.10 ,	0.21	IFN-γ	0	**-0.22**	**-0.37 ,**	**-0.07**
	0.5	0.07	-0.11 ,	0.24		0.5	**0.86**	**0.79 ,**	**0.93**
IL-6	0	**-0.17**	**-0.30 ,**	**-0.04**	TNF-α	0	**-0.17**	**-0.26 ,**	**-0.07**
	0.5	**-0.66**	**-0.84 ,**	**-0.49**		0.5	-0.13	-0.26 ,	0.00
IL-8pro	0	-0.08	-0.21 ,	0.07	GM-CSF	0	**-0.24**	**-0.45 ,**	**-0.05**
	0.5	**1.54**	**1.35 ,**	**1.71**		0.5	**-0.43**	**-0.58 ,**	**-0.28**

Significant results are in bold.

b. Causal MD and 95% CI's for chemokines over 2 time points.

Marker	Time	MD	95% CI		Marker	Time	MD	95% CI	
Eotaxin	0	**0.12**	**0.02 ,**	**0.23**	TARC	0	-0.01	-0.11 ,	0.09
(CCL11)	0.5	**0.34**	**0.24 ,**	**0.45**	(CCL17)	0.5	**-0.37**	**-0.43 ,**	**-0.31**
MCP-1	0	**0.10**	**0.04 ,**	**0.17**	BLC/BCA1	0	0.08	-0.02 ,	0.18
(CCL2)	0.5	**0.11**	**0.06 ,**	**0.17**	(CXCL13)	0.5	0.07	-0.05 ,	0.20
MCP-4	0	-0.04	-0.10 ,	0.02	IL-8	0	0.02	-0.14 ,	0.16
(CCL13)	0.5	**-0.18**	**-0.23 ,**	**-0.12**	(CXCL8)	0.5	**1.69**	**1.49 ,**	**1.89**
MIP-1β	0	**0.13**	**0.00 ,**	**0.26**	IP-10	0	**0.23**	**0.13 ,**	**0.34**
(CCL4)	0.5	-0.09	-0.25 ,	0.06	(CXCL10)	0.5	**1.19**	**1.10 ,**	**1.28**

Significant results are in bold.

**c. Causal MD and 95% CI's for soluble receptors over 2 time points.**

Marker	Time	MD	95% CI		Marker	Time	MD	95% CI	
sIL-2Rα	0	-0.03	-0.08 ,	0.03	sCD14	0	-0.03	-0.07 ,	0.01
	0.5	**0.38**	**0.33 ,**	**0.43**		0.5	**0.08**	**0.03 ,**	**0.12**
sIL-6R	0	0.11	-0.07 ,	0.29	sCD27	0	-0.03	-0.10 ,	0.03
	0.5	0.18	-0.02 ,	0.39		0.5	**0.24**	**0.17 ,**	**0.31**
sTNF-R2	0	-0.08	-0.14 ,	-0.02	sGP130	0	-0.03	-0.05 ,	0.01
	0.5	**0.31**	**0.25 ,**	**0.37**	(CD130)	0.5	-0.01	-0.04 ,	0.02

Significant results are in bold.
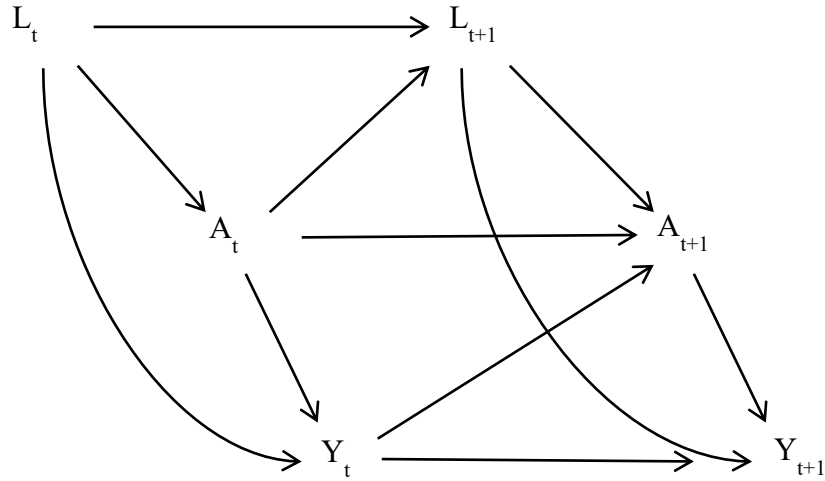
Figure 1. Directed acyclic graph of the study showing the relationships between exposure (A), outcome (Y) and confounding (L) variables over time (t).

Exposure (A) is ED drugs and outcome (Y) is the immune biomarker. Time-varying confounding variable (L) is the set of variables listed in Table 1. Time (t) is the study visits 1 to 5 in the HIV positive group and visits 1 to 2 in the HIV negative group. In the causal intermediate path from A_t to Y_{t+1} through L_{t+1}, L_{t+1} to Y_{t+1} path is also on a biased path from A_{t+1} to Y_{t+1} through L_{t+1}. Other confounding variables of A on Y relationship at each time point was left out of the graph for convenience.
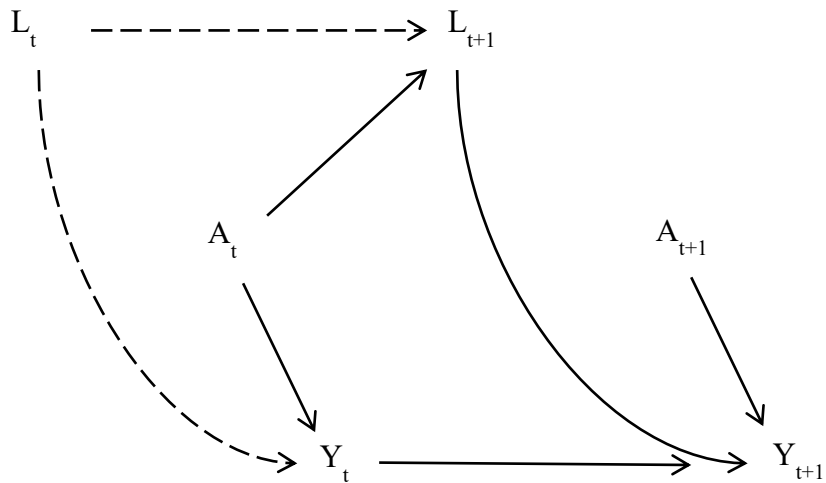
Figure 2. Directed acyclic graph of the study after intervention on the exposure (A) at each time point (t).

After randomizing on A_t and A_{t+1} (assigning ED drugs), biasing paths from A_t and A_{t+1} are removed, while causal intermediate paths from A_t to Y_{t+1} through L_{t+1} is preserved. Solid arrow lines represent the causal pathways of interest. Dashed arrow lines represent unbiased pathways after intervention on A. Pathways from other confounding variables to Y_t and Y_{t+1} were left out of the graph for convenience.

a. Comparison of ED drug users and non-user among HIV positive subjects by causal mean differences and 95% confidence intervals over four time points.
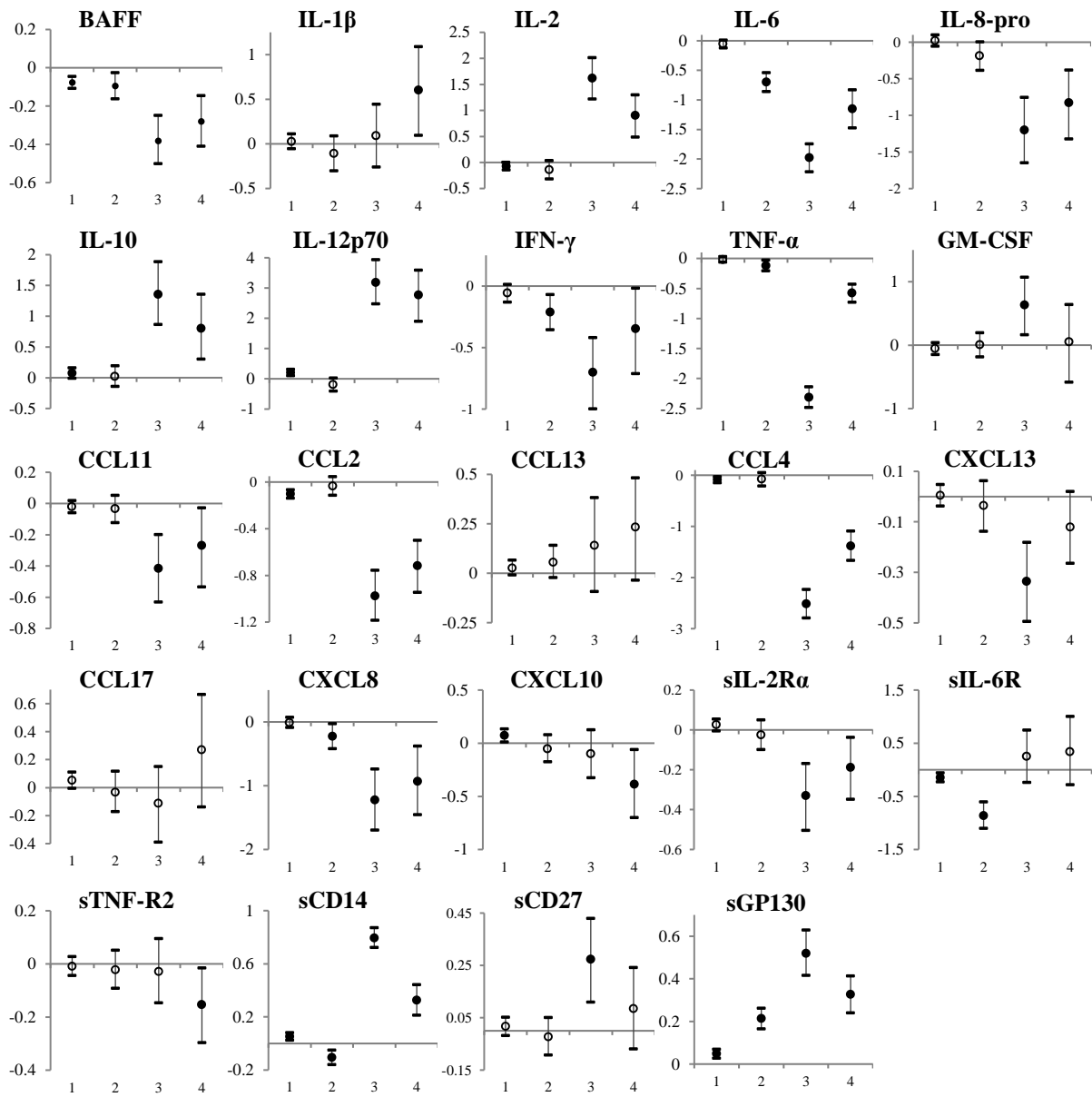
b. Comparison of ED drug users and non-user among HIV negative subjects by causal mean differences and 95% confidence intervals over two study visits.
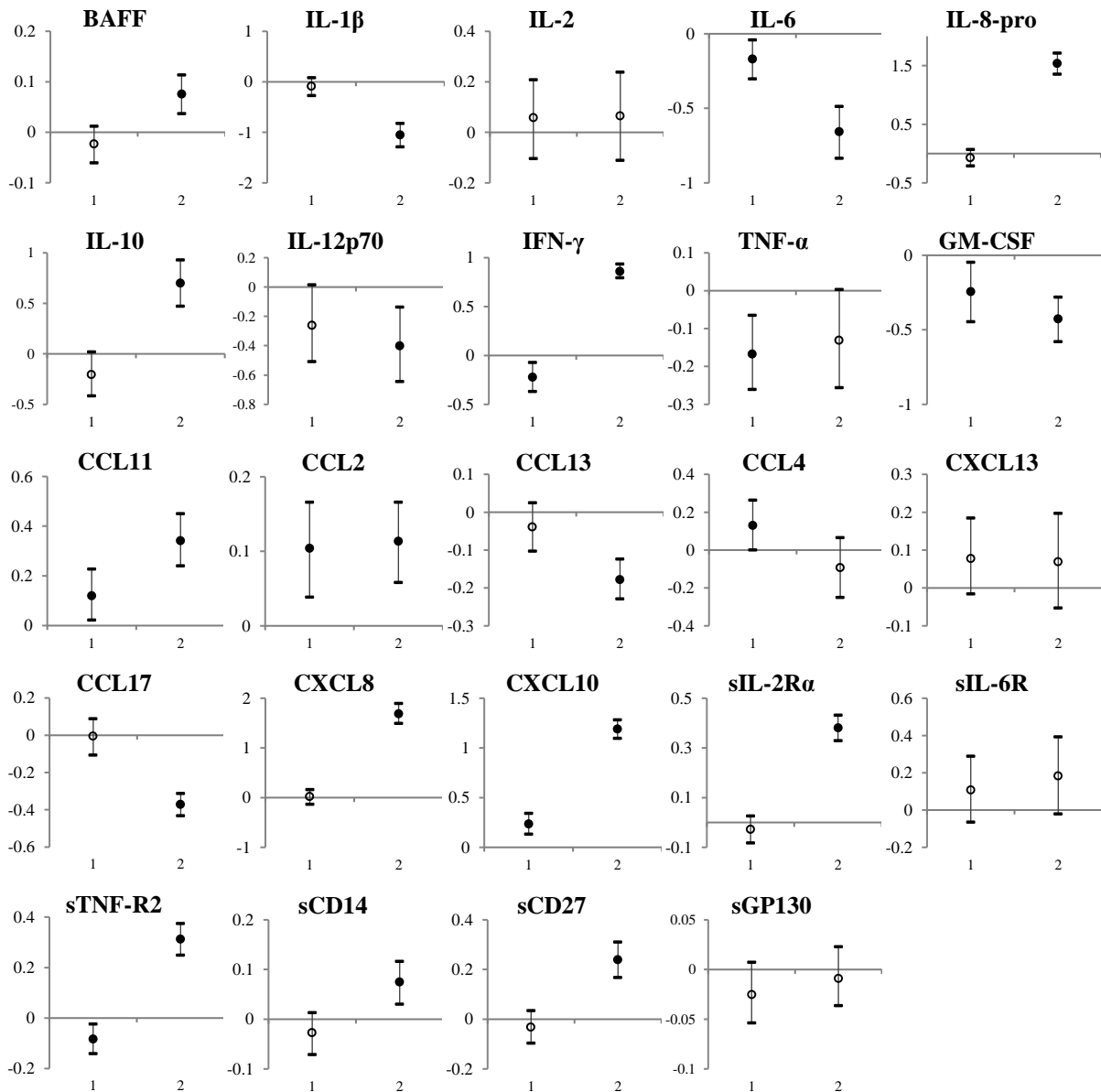
Figure 3.

a. Comparison of ED drug users and non-user among HIV positive subjects by causal mean differences and 95% confidence intervals over four time points.
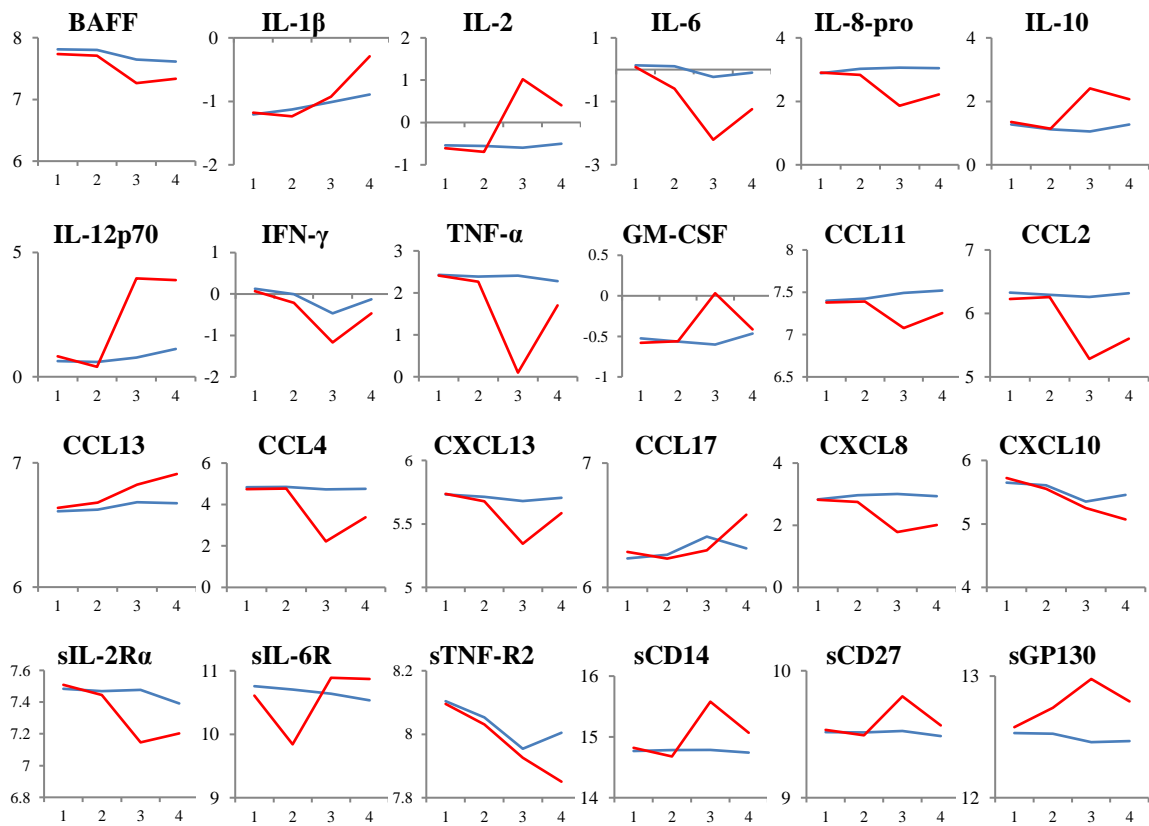
Horizontal axis shows the ARRA-1 study visits (1-4), where visit 1 is the baseline and visit 4 represents 1.5 years of follow-up. Vertical axis represents log-transformed outcomes. Filled circles represent statistically significant causal MDs that were computed by marginal structural models using variables in Table 1.

b. Comparison of ED drug users and non-user among HIV negative subjects by causal mean differences and 95% confidence intervals over two study visits.
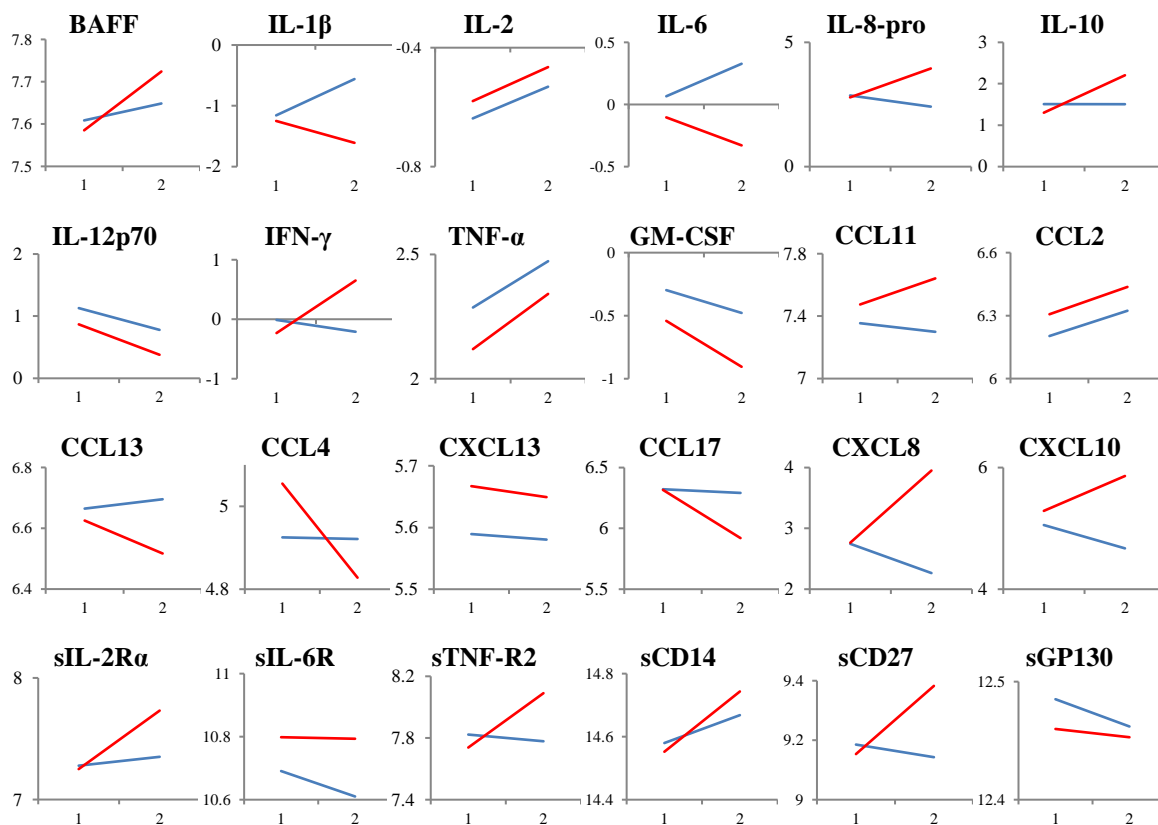
Horizontal axis shows the ARRA-1 study visits, where visit 1 is the baseline and visit 2 represents 6 months of follow-up. Vertical axis represents log-transformed outcomes. Filled circles represent statistically significant causal MDs that were computed by marginal structural models using variables in Table 1.

a. Mean levels of immune markers over time comparing ED drug users and non-users in HIV positive subjects.

b. Mean levels of immune markers over time comparing ED drug users and non-users in HIV negative subjects.

Figure 4.

    a. Mean levels of immune markers over time comparing ED drug users and non-users in HIV positive subjects.

Horizontal axis shows the MACS study visits (1-4), where visit 1 is the baseline and visit 4 represents 1.5 years of follow-up. Vertical axis represents log-transformed outcomes. Red solid lines represent ED drug users and blue solid lines show non-users. Mean levels are computed by potential outcomes from marginal structural models using variables in Table 1.

    b. Mean levels of immune markers over time comparing ED drug users and non-users in HIV negative subjects.

Horizontal axis shows the MACS study visits, where visit 1 is the baseline and visit 2 represents 6 months of follow-up. Vertical axis represents log-transformed outcomes. Red solid lines represent ED drug users and blue solid lines show non-users. Mean levels are computed by potential outcomes from marginal structural models using variables in Table 1.

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CHAPTER 6

Conclusion

Summary

The overarching purpose of this research was to examine the effect of using erectile dysfunction (ED) drugs on the immune capacity and function in both HIV infected and uninfected men who have sex with men (MSM). Animal studies in the past have provided some evidence of the biological plausibility in connecting ED drugs with CD4 and CD8 T cells, as well as various other markers of immune activation and inflammation. ED drugs are a selective and potent inhibitor of phosphodiesterase type 5 (PDE-5), which inhibits the degradation of cyclic guanosine monophosphate (cGMP) to GMP resulting in increased levels of cGMP. This then acts to modulate various immune responses and levels of inflammation.

To approach this research question, understanding the relationship between ED drug use and various demographic and behavorial factors in individuals in the Multicenter AIDS Cohort Study (MACS) study was necessary. The association between ED drug use and smoking, alcohol consumption, BMI, and other health condition factors were well established using cross-sectional studies in the literature. High-risk sexual activities, such as unprotected anal intercourse, has also been frequently documented with increased ED drug use. Use of recreational drugs with ED drugs were cited in several studies in the past, and also there were longitudinal studies showing a relationship between the two drugs. Using a bivariate random-intercept model, results from our study examining longitudinal patterns of ED drug use agreed with previous findings in the literature. We provided evidence of positive relationships between ED drug use and other recreational drugs, as well as sexual activities in both HIV positive and negative MSM on a population level over time. This suggests that on average, the more the ED drug were used in men, the use of recreational drugs and engagement of high-risk sexual activities also increased. In addition, we provided new

evidence that these positive correlations were consistently shown at an individual level in HIV positive men.

The independent effect of ED drugs on the immune cells were examined using the factors identified in the previous study as important time-varying confounding variables. Due the complex longitudinal nature of the MACS study, we applied a marginal structural model in the form of g-computation to account for multiple time-varying exposures, confounders, and outcomes. Re-creating counterfactual outcomes from baseline onwards with respect to ED drug use allowed us to examine the effect of taking ED drugs consistently over several time points. Our results indicated that ED drug use over time increased the immune capacity in HIV positive men, as shown by the higher CD4 cell counts and percentages compared to non-users. HIV negative men did not show the same results with ED drug use.

Assessment of immune markers using g-computation also showed that ED drugs generally dampened the inflammation levels in both HIV positive and negative men. Classic pro-inflammatory markers including IL and TNF-α, are elevated in response to inflammatory stimuli and the levels of these markers were mostly reduced in men who reported use of ED drugs throughout all time points. Conversely, anti-inflammatory markers such as IL-2, IL-10 and IL-12p70, prevent inflammatory responses and promote T regulatory cells. Interestingly, levels of anti-inflammatory markers were estimated to be higher in ED drug users over time than non-users in both groups of men, with the exception of IL-12p70 in HIV negative men. The results from this study showed positive results in markers of immune activation and inflammation with ED drug use over time, particularly in HIV positive men, which suggests increased T cell activation, improved immunity and reduced inflammation.

Residual inflammation in HIV positive men have been linked to disease progression and persistence of HI V even after the suppression of viral load in the presence of HAART.

Elimination of this residual inflammation has been a key focus in clinical care of HIV and the findings from this study may suggest ED drugs could be potentially used in addition to other agents to suppress the immune response and to dampen the residual inflammation in HIV positive men.