Title

Effects of Erectile Dysfunction Drugs Use on T-Cells and Immune Markers on Men Who Have Sex with Men

Journal

International Journal of Sexual Health, 34(3)

ISSN

1931-7611

Authors

Park, Jee Won
Arah, Onyebuchi A
Martinez-Maza, Otoniel
et al.

Publication Date

2022-07-03

DOI

10.1080/19317611.2022.2084200

Peer reviewed

International Journal of Sexual Health

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/wijs20

Effects of Erectile Dysfunction Drugs Use on T-Cells and Immune Markers on Men Who Have Sex with Men

Jee Won Park, Onyebuchi A. Arah, Otoniel Martinez-Maza, Adrian S. Dobs, Ken S. Ho, Frank J. Palella, Eric C. Seaberg & Roger Detels

To cite this article: Jee Won Park, Onyebuchi A. Arah, Otoniel Martinez-Maza, Adrian S. Dobs, Ken S. Ho, Frank J. Palella, Eric C. Seaberg & Roger Detels (2022) Effects of Erectile Dysfunction Drugs Use on T-Cells and Immune Markers on Men Who Have Sex with Men, International Journal of Sexual Health, 34:3, 462-473, DOI: 10.1080/19317611.2022.2084200

To link to this article: https://doi.org/10.1080/19317611.2022.2084200

INTERNATIONAL JOURNAL OF SEXUAL HEALTH
2022, VOL. 34, NO. 3, 462–473
https://doi.org/10.1080/19317611.2022.2084200

Effects of Erectile Dysfunction Drugs Use on T-Cells and Immune Markers on Men Who Have Sex with Men

Jee Won Park[a], Onyebuchi A. Arah[a], Otoniel Martinez-Maza[b], Adrian S. Dobs[c], Ken S. Ho[d], Frank J. Palella[e], Eric C. Seaberg[f] and Roger Detels[a]

[a]Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, CA, USA; [b]David Geffen UCLA School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA; [c]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [d]Department of Medicine, University of Pittsburgh, Pittsburgh, PA, USA; [e]Division of Infectious Diseases, Feinberg School of Medicine of Northwestern University, Chicago, IL, USA; [f]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

ABSTRACT

Objective: Examine prospective relationships between erectile dysfunction (ED) drugs and CD4 and CD8 T-cells, and immune markers among men who have sex with men (MSM).

Methods: Data from Multicenter AIDS Cohort Study, an observational prospective cohort study, with semiannual follow-ups conducted in four U.S. centers from 1998 onwards was used. Marginal structural models using g-computation were fitted to estimate the mean differences for the effects of self-reported ED drug use on CD4 and CD8 T-cell outcomes and immune biomarkers.

Results: Total of 1,391 men with HIV (MWH) and 307 men without HIV (MWOH) was included. Baseline mean CD4 cell count among MWH and MWOH was 499.9 and 966.7 cells/μL, respectively. At baseline, 41.8% of MWH were virally suppressed. ED drug users reported a mean of 44.4 months of exposure to ED drugs. ED drug use was associated with increased CD4 cell outcomes among MWH but not MWOH. Mean differences in CD4 cell counts after 1 year of ED drug use was 57.6 cells/μL and increased to 117.7 after 10 years among MWH. CD8 counts were higher in ED drug users among MWH over 10 years than non-users; no consistent differences were found among MWOH. ED drug use appeared to reduce immune marker levels, such as IL-6 and increase markers, such as IL-10. We observed similar effects of ED drug use on biomarker levels among MWOH.

Conclusion: Long-term use of ED drugs do not adversely affect immune function among MWH or MWOH. Future studies on the relationships between different types of ED drugs and effects on T-cell subtypes are warranted.

ARTICLE HISTORY
Received 30 December 2021
Revised 17 April 2022
Accepted 22 May 2022

KEYWORDS
Cohort studies; phosphodiesterase 5 inhibitors; men who have sex with men (MSM); T-lymphocytes; biomarkers

Introduction

Erectile dysfunction (ED) drugs are commonly used by men who have sex with men (MSM) for erectile dysfunction treatment and recreational purposes (Fisher et al., 2006; Harte & Meston, 2011; Kim et al., 2002). ED drugs were evaluated for treatment of angina, ED, and pulmonary arterial hypertension. As a selective and potent inhibitor of phosphodiesterase type 5 (PDE-5), which increases cyclic guanosine monophosphate (cGMP) levels, ED drugs were found to be effective for treating ED by increasing blood flow to the penis (Barnett & Machado, 2006). Treatment with PDE-5 inhibitors has been shown to be effective among individuals with pulmonary arterial hypertension, but there is also some evidence pointing to other conditions, such as coronary arterial disease and hypertension by relaxing vessel walls, resulting in increased blood flow (Barnes et al., 2019; Chrysant, 2013; Kloner, 2004; Wada et al., 2016).

Despite the common use of ED drugs, their effects on the human immune system remain unclear. Few studies have examined the impact of ED drug use on the immune system. A potential mechanism by which ED drugs could affect

CONTACT Roger Detels detels@ucla.edu Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles, 650 Young Drive South, Los Angeles, CA 90095-1772, USA.

Supplemental data for this article is available online at https://doi.org/10.1080/19317611.2022.2084200

immune cells and markers might be through control of cGMP levels by preventing cGMP degradation to GMP. Inhibition of cGMP degradation could then act to regulate the activities of immune cells (Kniotek & Boguska, 2017). In animal studies, the use of ED drugs, mainly those containing sildenafil, showed immunomodulatory effects in healthy mice (Karakhanova et al., 2013; Szczypka & Obmińska-Mrukowicz, 2010). For example, one study showed increased survival of tumor-bearing mice when sildenafil was administered (Serafini et al., 2006), while another study reported the effects of sildenafil on healthy human lymphocytes *in vitro* and demonstrated beneficial immunomodulatory effects (Pifarre et al., 2014).

Evidence is more limited regarding the potential immunomodulatory effects of ED drugs in humans. Furthermore, no studies have examined the longitudinal impact of ED drugs on the immune system among MSM. The Multicenter AIDS Cohort Study (MACS) has collected information and data on immune markers of MSM since 1983. Thus, the MACS presented an opportunity to do a retrospective long-term study on immune markers and the use of erectile dysfunction drugs in men. Therefore, our study aimed to: 1) estimate the longitudinal effects of ED drug use on CD4 and CD8 cells; and 2) examine how ED drug use changes the levels of immune biomarkers among MSM.

Methods

Study population

Participants were from the MACS, an observational semiannual study of MSM with or without HIV at four sites in the United States (Baltimore, Chicago, Pittsburgh, and Los Angeles) (Detels et al., 1992). Follow-up visits were scheduled every six months covering events and medications used since their last visit. Participants who had been active in the study beginning in 1998 were included since the FDA approved ED drugs in 1998. Hence, the baseline time for men with HIV (MWH) was defined as the first MACS visit in 1998 while seropositive, or the first MACS visit after HIV seroconversion. The baseline for men without HIV (MWOH) was defined as the first visit during or after 1998 while seronegative.

To examine immune markers, data were used from ARRA1 (American Recovery and Reinvestment Act, study 1), a sub-study of the MACS. The purpose of the ARRA1 was to examine the levels of markers among all MACS HIV seroconverters and highly-active antiretroviral therapy (HAART) initiators during follow-up. ARRA1 included serum samples from MACS in-person visits that were most proximal to the time of HIV seroconversion and visits most proximal to HAART initiation (before and after) for all HAART users. Samples from MWOH who were of similar age and race were also included.

The MACS study was approved by the Institutional Review Board (IRB) at the participating institutions at each study site, and all participants provided their written informed consent to participate in the study .

Measures

The exposure variable was a binary self-reported ED drug use (yes/no) since the previous MACS visit, which included sildenafil, tadalafil, and vardenafil. The use of ED drugs was regardless of the purpose, i.e., the binary ED drug use variable informed whether the use was for the treatment of ED or for recreational use.

Covariates included age, race/ethnicity, education, smoking, alcohol consumption, obesity, and study center attended. Other variables included dichotomized (yes/no) self-reported use of marijuana, poppers, stimulants, testosterone, depression medication, and antiretroviral therapies (ART) since the last visit. Health-related factors included hepatitis C virus (HCV) infection, other comorbidities (stroke, congestive heart problems, prostate cancer/surgery, bladder cancer/surgery), using ICD-9 codes, and log-transformed HIV RNA (viral load). Number of men with whom the participants engaged in unprotected insertive anal intercourse (UIAI) since the last visit ($\geq$1/0) and the number of male and female sex partners ($\geq$2/0–1) were also included. Covariates were selected based on prior studies and evidence of host-factor influences on biomarker levels (McKay et al., 2016; Wada et al., 2015).

Outcome variables for the estimation of the longitudinal effects of ED drug use on CD4 and CD8 cells (first aim) included CD4 and CD8 cell counts, CD4 percentages of total lymphocytes (CD4%), and CD4:CD8 cell ratios, which were measured from blood samples taken during visits. CD4 and CD8 cell measures from the previous visit were also included as time-varying covariates in the analysis of CD4 and CD8 cell outcomes, respectively. For the examination of how ED drug use changes the levels of immune biomarkers among MSM (second aim), 24 immune markers classified into three categories (cytokines, chemokines, and soluble receptors) were examined, one at a time, as the outcome variable. Detailed information on the measurements of immune markers has been described elsewhere (Wada et al., 2015, 2016). Immune markers with measures below the lower limit of detection (LLD) were assigned a value equal to the midpoint between the LLD and zero. All outcome variables were log-transformed for analysis.

Statistical analysis

Participants in the study were divided into two groups by HIV serostatus. Descriptive statistics were used to compare participant baseline characteristics by serostatus and ED drug use. The mean number of visits at which ED drugs were used during the previous visit was calculated for each user. Given that the MACS is a longitudinal study that included time-varying exposures, confounders, and outcomes, we used g-computation to estimate the effects of ED drug use on levels of immune cells and markers among MWH and MWOH (Robins, 1986; Robins et al., 2000). This longitudinal design is shown in the simplified directed acyclic graph (DAG) in Supplemental Figure 1. In such longitudinal settings, adjustments for time-varying confounding variables (L_{t+1}, where t is the MACS visit) were necessary to estimate the effects of subsequent exposures (A_{t+1}) without blocking their indirect mediating role on the path from previous exposure (A_t) to the later outcome (Y_{t+1}), in which case they should not be adjusted (Hernan et al., 2000; Hernán et al., 2001; Robins et al., 2000). Using marginal structural models, such as g-computation, we eliminated such time-varying confounding and preserved the ability to discern the causal mediating paths. The simplified DAG after intervention on exposure at each time point is shown in Supplemental Figure 2. Confounding by the time-varying variables was eliminated, while their mediating pathways from previous exposure to the subsequent outcome remained.

To estimate the average effects of ED drug use over time on subsequent outcomes, we used Monte Carlo simulations with 1,000 repetitions to execute the g-computation analyses and to estimate the effects. This entailed simulating the data scenario in the intervention DAG shown in Supplemental Figure 2 from our observed data, assuming the pre-intervention DAG in Supplemental Figure 1. First, we obtained the distribution of the binary ED drug use at each visit and used the observed distribution of ED drug to randomly assign the binary exposure intervention A (i.e., ED drug use) at each time t (i.e., MACS visit) to estimate subsequent potential outcomes. Second, we fitted flexible models of each of A's sequential consequences, namely the outcomes Y's (i.e., CD4, CD8, or immune markers) and the set of time-varying covariates L's, and obtained the regression parameters. Product terms between ED drug use and time-fixed variables, such as age, race/ethnicity, and education were also included in the model. Product terms between ED drug use and other confounding variables were evaluated but were found to be statistically non-significant, and were therefore not included in the model. Third, we used the regression parameters from the second step and the simulated binary A's from the first step to generate new time-varying covariates (L's) and new potential outcomes (Y's) as our new potential mediating and outcome variables devoid of time-varying confounding by the new L's. Fourth, we used the simulated ED drug use (A's) and outcomes (Y's) to estimate the causal mean differences for averaged effects of ED drug use *vs.* no ED drug use at each time point on subsequent outcomes (Y). Confidence limits were computed by summarizing the results over the 1,000 repetitions, using the 2.5th and 97.5th percentiles as the lower and upper confidence limits.

Table 1. Descriptive Baseline Characteristics among MWH and MWOH in the MACS Who Reported ED Drug Use Since Last Visit Compared to Those Who Did Not Report ED Drug Use Since Last Visit.

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

BMI: body mass index; HAART: highly antiretroviral therapy; HCV: hepatitis C virus; UIAI: unprotected insertive anal intercourse.
*Mean ± s.d.
†Include cocaine, ecstasy, methamphetamine, and uppers.
‡Include stroke, coronary heart failure, prostate surgery/cancer, bladder surgery/cancer.

All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics

Data were available for 1,636 men with a total of 36,095 observations. Some men in the study contributed to both the MWH and MWOH groups, so 1,391 MWH with 29,343 observations and 307 MWOH with 6,752 observations were included in the analyses. MWH had an average of 21.1 ± 11.3 study visits, and MWOH had 22.0 ± 9.7 visits (see Supplemental Table 1). The mean number of visits at which users reported ED drug use in the previous 6 months (visit) was 7.4, or a minimum of 44.4 months (7.4 × 6 months) of accumulated reported exposure to ED drugs.

Table 1 shows the baseline characteristics of the MACS participants. At baseline, 9.6% of MWOH reported ED drug use, compared to 6.0% of MWH. The mean age of MWH was 42.5 ± 7.8 years, and MWOH was 44.8 ± 9.5 years, with a higher mean age among ED drug users compared to non-users. Mean CD4 at baseline for MWH was higher among ED drug users (553.0 ± 32.9 *vs.* 495.6 ± 8.1 cells/μL). Among MWOH, mean CD4 counts were similar between users and non-users (961.1 ± 300.2 *vs.* 967.3 ± 319.0 cells/μL). ED drug users had mean CD4 cell percentages similar to non-users, regardless of HIV serostatus. Mean CD8 counts among MWH were higher among non-users, while ED drug users among MWOH had higher mean CD8 counts. Mean CD4:CD8 ratios were close to 0.5 for MWH and 2.0 for MWOH. Demographic characteristics of MWH and MWOH in ARRA1 were similar to those observed in the MACS (Table 2).

Immune cell outcomes

The causal mean differences comparing ED drug users and non-users in the outcome fluctuated for five time points [years 0 (baseline), 1, 2, 5, and 10], but were consistently higher among ED drug users. In Table 3, the mean CD4 count among MWH was higher among ED drug users at baseline than non-users (mean difference, 107.3 [95% CL: 73.3, 141.5]) and remained higher, although with lesser differences for ED

Table 2. Descriptive Baseline Characteristics among MWH and MWOH 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.

	MWH ED drug use		MWOH ED drug use	
	No ($n = 1,053$) *N* (%)	Yes ($n = 95$) *N* (%)	No ($n = 244$) *N* (%)	Yes ($n = 42$) *N* (%)
Age*	43.3 ± 7.8	46.8 ± 8.3	47.0 ± 9.7	49.2 ± 9.2
Non-white	449 (42.6)	35 (36.8)	119 (48.8)	16 (38.1)
Education (college or higher)	527 (50.1)	48 (50.5)	124 (50.8)	24 (57.1)
Smoker	388 (36.9)	38 (40.0)	105 (43.0)	13 (31.0)
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)	50 (20.5)	9 (21.4)
Marijuana	394 (37.4)	42 (44.2)	65 (26.6)	12 (28.6)
Poppers	272 (25.8)	42 (44.2)	50 (20.5)	14 (33.3)
Stimulants†	211 (20.0)	32 (33.7)	56 (23.0)	10 (23.8)
Depression medication	250 (23.7)	34 (35.8)	40 (16.4)	10 (23.8)
On testosterone	47 (4.5)	11 (11.6)		
HAART	601 (57.1)	43 (45.3)		
HCV infection	104 (9.9)	13 (13.7)	50 (20.5)	9 (21.4)
Pre-existing conditions‡	60 (5.7)	7 (7.4)	14 (5.7)	2 (4.8)
Sex partners (≥2 partners)	425 (40.4)	60 (63.2)	107 (43.9)	31 (73.8)
UIAI (≥1 partner)	213 (17.3)	34 (42.0)	66 (27.1)	18 (42.9)
Study site (center)				
Baltimore	257 (24.4)	26 (27.4)	53 (21.7)	12 (28.6)
Chicago	262 (24.9)	26 (27.4)	55 (22.5)	12 (28.6)
Pittsburgh	216 (20.5)	15 (15.8)	87 (35.7)	13 (31.0)
Los Angeles	318 (30.2)	28 (29.5)	49 (20.1)	5 (11.9)

BMI: body mass index; HAART: highly active antiretroviral therapy; HCV: hepatitis C virus; UIAI: unprotected insertive anal intercourse.
*Mean ± s.d.
†Include cocaine, ecstasy, methamphetamine, and uppers.
‡Include stroke, coronary heart failure, prostate surgery/cancer, bladder surgery/cancer.

Table 3. Causal Mean Differences and 95% Confidence Limits (CL) for the Effects of ED Drug Use on CD4 and CD8 Cell Outcomes among MWH and MWOH at 0, 1, 2, 5, and 10 Years of Follow-up.

		MWH		MWOH	
	Year	Mean difference	95% CL	Mean difference	95% CL
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

Note: Causal mean differences were calculated using g-computation over 1,000 Monte Carlo simulations. Confidence limits were calculated by summarizing over 1,000 simulations and reported the 2.5th and 97.5th percentiles.

drug use over time. Year 1 mean difference was 57.6 (95% CL: 17.8, 94.8); year 2, 56.4 (95% CL: 15.2, 98.3); and year 5, 28.0 (95% CL: −21.6, 85.0). However, the differences increased again to 117.7 (95% CL: 37.1, 197.4) after 10 years. Causal mean difference for MWOH at baseline was −43.7 (95% CL: −91.9, 3.0) but was higher for non-users −55.9 (95% CL: −106.7, −4.6) after 10 years. When CD8 cell counts were the outcome, the causal mean difference at baseline was −23.1 (95% CL: −60.4, 12.4), but became positive (95.3 [95% CL: 18.7, 169.5]) after ten years

among MWH. Similar changes at ten years were observed among MWOH (compared to MWH) at baseline (−29.4 [95% CL: −69.2, 10.8]) and at ten years (47.0 [95% CL: −2.7, 105.1]). Among MWH, CD4 cell percentages were higher at baseline among ED drug users compared to ED drug non-users and remained higher over time. Among MWOH, CD4 cell percentage differences between ED drug users and non-users were not apparent until later in the course of observation. The average differences by ED drug use in CD4:CD8 ratios remained constant over time among MWH. In contrast, among MWOH, ED drug non-users had higher ratios after their seventh MACS visit than the users. Supplemental Figure 3 illustrates the mean levels of CD4 and CD8 cell outcomes among ED drug users and non-users over time.

Immune biomarker outcomes

Tables 4 and 5 present the results for levels of immune markers among MWH and MWOH in ARRA1, respectively. The causal mean differences for the levels of five inflammatory cytokines (BAFF, IL-6, IL-8, IFN-γ, and TNF-α) indicated lower levels associated with ED drug use over time, except for IL-1β levels, which were higher after the third observation (Table 4a). Levels of anti-inflammatory cytokines IL-2, IL-10, and IL-12p70 were higher among ED drug users than non-users over time. Mean levels of chemokines, except CCL17, were lower with ED drug use over time (Table 4b). Mean differences for CCL17 fluctuated. Mean levels of four soluble receptors were slightly higher among ED drug users, but sIL-2Rα and sTNF-R2 levels were higher among non-users (Table 4c).

Among MWOH, mean levels of four pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, GM-CSF) were lower among ED drug users than non-users (Table 5a). Mean levels of other pro-inflammatory markers (BAFF, IL-8, IFN-γ) were higher among ED drug users. Mean anti-inflammatory biomarker levels of IL-2 and IL-10 were estimated to be higher among ED drug users after the second observation. Mean levels of five chemokines (CCL11, CCL2, CXCL13, CXCL8, CXCL10) were higher among ED drug users (Table 5b), and five soluble receptors (sIL-2Rα, sIL-6R, sTNF-R2, sCD14, sCD27) were also higher among ED drug users (Table 5c). Supplemental Figure 4 illustrates the mean outcome levels among ED drug users and non-users over time.

Discussion

In this prospective study of men who participated in the MACS, ED drug use was associated with differences in levels of CD4 and CD8 T-cells over time among both MWH and MWOH. In particular, accumulated ED drug use was associated with increases over time in the number of CD4 cells among MWH. CD8 cell counts were also higher among MWH ED drug users with over ten years of use than non-users, perhaps reflecting residual inflammation in these men, whereas almost no significant differences for ED drug use were observed among MWOH. The CD4:CD8 cell ratios generally remained stable over time, but were higher among MWH ED drug users than non-users. Overall, our findings suggest immunomodulatory effects of ED drug use on markers of immune activation and inflammation among MSM. Among MWH, mean levels of five pro-inflammatory cytokines (BAFF, IL-6, IL-8, IFN-γ, TNF-α) were lower among ED drug users than non-users. At the same time, levels of an anti-inflammatory cytokine (IL-10) and two cytokines associated with enhanced T-cell immunity (IL-2, IL-12p70) were higher. Mean levels of most of the chemokines and some of the soluble receptors evaluated were also lower among ED drug users than non-users. Levels of several important markers changed in a favorable direction: IL-1β, IL-6, and TNF-α levels decreased, while IL-10 increased. Similar findings were not observed among MWOH.

Findings from this study are similar to those reported previously for animal studies that administered ED drugs, mainly sildenafil, on CD4 and CD8 cells. One study reported significantly higher naïve CD4 cells and lower central memory CD8 cells when healthy male mice were treated with sildenafil (Karakhanova et al., 2013); meanwhile, another study showed increased CD8 cells (Szczypka & Obmińska-Mrukowicz, 2010).

Table 4. Causal Mean Differences and 95% Confidence Limits (CL) for the Effects of ED Drug Use on Immune Biomarkers among MWH Across 5 Study Visits.

a. Causal mean differences and 95% CLs for cytokines over 5 time points

Marker	Time	Mean difference	95% CL	Marker	Time	Mean Difference	95% CL
BAFF	0	−0.08	−0.11, −0.05	IL-10	0	0.07	−0.01, 0.16
	1	−0.10	−0.16, −0.03		1	0.02	−0.14, 0.19
	2	−0.38	−0.50, −0.25		2	1.36	0.86, 1.88
	3	−0.28	−0.41, −0.15		3	0.80	0.30, 1.35
	4	0.26	−0.32, 0.86		4	0.72	−0.10, 1.50
IL-1β	0	0.03	−0.05, 0.11	IL-12p70	0	0.20	0.09, 0.31
	1	−0.11	−0.30, 0.09		1	−0.19	−0.41, 0.02
	2	0.09	−0.26, 0.44		2	3.18	2.47, 3.93
	3	0.60	0.09, 1.09		3	2.77	1.89, 3.58
	4	1.67	0.41, 2.99		4	2.27	0.38, 4.21
IL-2	0	−0.07	−0.14, 0.00	IFN-γ	0	−0.06	−0.13, 0.01
	1	−0.14	−0.32, 0.03		1	−0.21	−0.36, −0.07
	2	1.62	1.22, 2.01		2	−0.70	−1.00, −0.42
	3	0.90	0.49, 1.29		3	−0.35	−0.71, −0.02
	4	0.96	−0.02, 1.97		4	0.12	−0.99, 1.19
IL-6	0	−0.06	−0.13, 0.01	TNF-α	0	−0.02	−0.06, 0.03
	1	−0.70	−0.86, −0.54		1	−0.12	−0.21, −0.03
	2	−1.98	−2.22, −1.75		2	−2.31	−2.48, −2.14
	3	−1.15	−1.47, −0.83		3	−0.58	−0.73, −0.43
	4	−0.91	−2.34, 0.59		4	−0.49	−0.77, −0.20
IL-8	0	0.02	−0.06, 0.10	GM-CSF	0	−0.06	−0.15, 0.04
	1	−0.19	−0.39, 0.00		1	0.00	−0.19, 0.19
	2	−1.20	−1.65, −0.75		2	0.63	0.16, 1.07
	3	−0.83	−1.33, −0.38		3	0.05	−0.58, 0.64
	4	−1.51	−2.40, −0.65		4	0.21	−2.14, 2.53

b. Causal mean differences and 95% CLs for chemokines over 5 time points

Eotaxin (CCL11)	0	−0.02	−0.06, 0.02	TARC (CCL17)	0	0.05	−0.01, 0.11
	1	−0.03	−0.12, 0.05		1	−0.03	−0.17, 0.12
	2	−0.42	−0.63, −0.20		2	−0.11	−0.39, 0.15
	3	−0.27	−0.53, −0.03		3	0.27	−0.14, 0.67
	4	0.28	−0.14, 0.73		4	−0.33	−1.04, 0.38
MCP-1 (CCL2)	0	−0.10	−0.14, −0.07	BLC/BCA1 (CXCL13)	0	0.00	−0.04, 0.05
	1	−0.03	−0.11, 0.05		1	−0.04	−0.14, 0.06
	2	−0.98	−1.19, −0.76		2	−0.34	−0.49, −0.18
	3	−0.72	−0.95, −0.50		3	−0.12	−0.26, 0.02
	4	−0.03	−0.79, 0.67		4	−0.47	−0.79, −0.17
MCP-4 (CCL13)	0	0.03	−0.01, 0.07	IL-8 (CXCL8)	0	−0.01	−0.09, 0.07
	1	0.06	−0.02, 0.14		1	−0.22	−0.42, −0.03
	2	0.14	−0.09, 0.38		2	−1.23	−1.70, −0.74
	3	0.23	−0.03, 0.48		3	−0.93	−1.46, −0.38
	4	0.30	−0.22, 0.81		4	−1.05	−1.99, −0.09
MIP-1β (CCL4)	0	−0.09	−0.15, −0.03	IP-10 (CXCL10)	0	0.07	0.01, 0.13
	1	−0.08	−0.21, 0.05		1	−0.05	−0.18, 0.08
	2	−2.51	−2.79, −2.23		2	−0.10	−0.33, 0.13
	3	−1.38	−1.66, −1.09		3	−0.39	−0.70, −0.06
	4	0.04	−0.45, 0.51		4	−0.45	−1.40, 0.52

c. Causal mean differences and 95% CLs for soluble receptors over 5 time points

sIL-2Rα	0	0.02	−0.01, 0.05	sCD14	0	0.05	0.02, 0.08
	1	−0.02	−0.10, 0.05		1	−0.11	−0.16, −0.05
	2	−0.33	−0.50, −0.17		2	0.79	0.72, 0.87
	3	−0.19	−0.35, −0.04		3	0.32	0.21, 0.44
	4	−0.33	−1.03, 0.38		4	0.84	−0.39, 2.08
sIL-6R	0	−0.15	−0.23, −0.06	sCD27	0	0.02	−0.02, 0.05
	1	−0.86	−1.11, −0.61		1	−0.02	−0.09, 0.05
	2	0.25	−0.24, 0.74		2	0.27	0.11, 0.43
	3	0.34	−0.29, 1.00		3	0.09	−0.07, 0.24
	4	−0.06	−0.70, 0.60		4	−0.14	−0.52, 0.24
sTNF-R2	0	−0.01	−0.04, 0.03	sGP130 (CD130)	0	0.05	0.03, 0.07
	1	−0.02	−0.09, 0.05		1	0.21	0.16, 0.26
	2	−0.03	−0.15, 0.10		2	0.52	0.42, 0.63
	3	−0.15	−0.30, −0.02		3	0.33	0.24, 0.41
	4	−0.22	−0.85, 0.42		4	−2.05	−3.08, −1.04

Note: Causal mean differences were calculated using g-computation over 1,000 Monte Carlo simulations. Confidence limits were calculated by summarizing over 1,000 simulations and reported the 2.5th and 97.5th percentiles.

Table 5. Causal Mean Differences and 95% Confidence Limits (CL) for the Effects of ED Drug Use on Immune Biomarkers among MWOH Across 2 Study Visits.

Marker	Time	Mean difference	95% CL	Marker	Time	Mean difference	95% CL
a. Causal mean differences and 95% CLs for cytokines over 2 time points							
BAFF	0	−0.02	−0.06, 0.01	IL-10	0	−0.21	−0.42, 0.02
	1	0.08	0.04, 0.11		1	0.70	0.47, 0.93
IL-1β	0	−0.09	−0.27, 0.08	IL-12p70	0	−0.26	−0.51, 0.01
	1	−1.05	−1.29, −0.82		1	−0.40	−0.65, −0.14
IL-2	0	0.06	−0.10, 0.21	IFN-γ	0	−0.22	−0.37, −0.07
	1	0.07	−0.11, 0.24		1	0.86	0.79, 0.93
IL-6	0	−0.17	−0.30, −0.04	TNF-α	0	−0.17	−0.26, −0.07
	1	−0.66	−0.84, −0.49		1	−0.13	−0.26, 0.00
IL-8	0	−0.08	−0.21, 0.07	GM-CSF	0	−0.24	−0.45, −0.05
	1	1.54	1.35, 1.71		1	−0.43	−0.58, −0.28
b. Causal mean differences and 95% CLs for chemokines over 2 time points							
Eotaxin (CCL11)	0	0.12	0.02, 0.23	TARC (CCL17)	0	−0.01	−0.11, 0.09
	1	0.34	0.24, 0.45		1	−0.37	−0.43, −0.31
MCP-1 (CCL2)	0	0.10	0.04, 0.17	BLC/BCA1 (CXCL13)	0	0.08	−0.02, 0.18
	1	0.11	0.06, 0.17		1	0.07	−0.05, 0.20
MCP-4 (CCL13)	0	−0.04	−0.10, 0.02	IL-8 (CXCL8)	0	0.02	−0.14, 0.16
	1	−0.18	−0.23, −0.12		1	1.69	1.49, 1.89
MIP-1β (CCL4)	0	0.13	0.00, 0.26	IP-10 (CXCL10)	0	0.23	0.13, 0.34
	1	−0.09	−0.25, 0.06		1	1.19	1.10, 1.28
c. Causal mean differences and 95% CLs for soluble receptors over 2 time points							
sIL-2Rα	0	−0.03	−0.08, 0.03	sCD14	0	−0.03	−0.07, 0.01
	1	0.38	0.33, 0.43		1	0.08	0.03, 0.12
sIL-6R	0	0.11	−0.07, 0.29	sCD27	0	−0.03	−0.10, 0.03
	1	0.18	−0.02, 0.39		1	0.24	0.17, 0.31
sTNF-R2	0	−0.08	−0.14, −0.02	sGP130 (CD130)	0	−0.03	−0.05, 0.01
	1	0.31	0.25, 0.37		1	−0.01	−0.04, 0.02

Note: Causal mean differences were calculated using g-computation over 1,000 Monte Carlo simulations. Confidence limits were calculated by summarizing over 1,000 simulations and reported the 2.5th and 97.5th percentiles.

An *in vitro* study of sildenafil-treated mice demonstrated decreased adaptive immune responses while regulatory T-cell functions were enhanced (Pifarre et al., 2014). Further, two other studies have also shown that tadalafil administration enhanced systemic immunity by increasing CD4 and CD8 T-cell proliferation (Califano et al., 2015; Weed et al., 2015). Thus, findings from both animal studies and our present study show that ED drug use has a potential impact on the immune capacity, i.e., ED drug use may change the levels of immune cells.

In our study, levels of CD8 cells were initially slightly lower among ED drug users, regardless of HIV serostatus, but after continuous ED drug use, CD8 counts were higher compared to non-users. Several factors may explain the observed changes in levels of CD8, including concurrent viral infections, residual inflammation associated with low-level HIV replication, and HAART use itself (Cao et al., 2016). Another possible explanation may be a sildenafil-induced restoration of CD8 cells, as previously demonstrated in tumor-bearing mice, when administering sildenafil partially restored T-cell receptors (Meyer et al., 2011; Serafini et al., 2006).

Levels of IL-6 appeared to decline over time, regardless of HIV serostatus. Higher IL-6 concentrations have been associated with untreated HIV infection, disease progression, and mortality (Deeks, 2011; Nixon & Landay, 2010; Siewe & Landay, 2018). In a study of healthy mice, sildenafil reduced levels of serum IL-6 (Karakhanova et al., 2013), and in patients with diabetes, chronic use of PDE-5 inhibitors demonstrated decreases in IL-6 by improving nitrous oxide production (Aversa et al., 2008; Santi et al., 2015). Levels of other important biomarkers, such as IL-1β, TNF-α, and IFN-γ, were also reduced in animal studies in which ED drugs were administered. In sildenafil-treated mice, TNF-α and IFN-γ were reduced, while another study demonstrated the same results with decreased IL-1β (Nunes et al., 2012, 2015; Pifarre et al., 2014; Yildirim et al., 2010). The estimated TNF-α levels were lower over time among ED drug users in our study, and we saw no differences until later among MWH in IL-1β levels when comparing ED drug users and non-users, which was also observed in other studies (Karakhanova et al., 2013; Szczypka & Obmińska-Mrukowicz, 2010).

Corroborative of our findings on the immune markers, levels of anti-inflammatory markers, such as IL-10, were observed to be higher in studies evaluating ED drugs (Karakhanova et al., 2013; Nunes et al., 2015). However, other studies have shown conflicting results, or shown no changes to IL-2 or IL-10 levels (Nunes et al., 2012, 2015; Pifarre et al., 2014; Szczypka et al., 2012). We observed that the levels of these two cytokines were higher among ED drug users regardless of HIV serostatus. IL-12p70 levels were higher among MWH, but not MWOH. The higher observed levels of anti-inflammatory (IL-10) or T-cell-activating (IL-2, IL-12p70) cytokines, regardless of HIV serostatus, among ED drug users may suggest an increased immune competence that is a consequence of ED drug use. Thus, ED drugs may exert a favorable anti-inflammatory response for conditions, such as inflammatory arthritis or the acute inflammation associated with SARS-CoV-2 infection.

Further, the potential anti-inflammatory effects of ED drug use among MSM may have clinical implications. As MWH grow older, they are more in need of and likely to use ED drugs. Consequently, over time, the prevalence of erectile dysfunction and ED drug use in this population are likely to increase. Our observation that long-term use of ED drugs do not adversely affect immune function among either MWH or MWOH is reassuring.

Limitations and strengths

Our study is not without limitations. First, we could not look directly at the frequency per month of ED drug use because they were not available, and analyses for different ED drugs and reasons for use could not be undertaken since that information was also unavailable. However, the ED drug users reported 44.4 months of observed and accumulated exposure to the drugs. It is unlikely that a serious adverse effect of ED drug use would be missed with that level of scrutiny. Further, the frequency per use or dose per use was unlikely to have varied among these group of men in the MACS. In addition, prescription data for ED drugs were not available for each participant, so self-reported ED drug use could not be confirmed. Nonetheless, future studies should use medical records for objective measures of ED drug use and explore the impact of varying frequencies and dosages of ED drugs on immune cells and markers. Second, it could be argued that ED drugs were more likely to be used by healthier and more physically active men with fewer adverse health conditions, although the fact that they were using ED drugs would suggest that they may have poorer health since they were taking drugs to restore their ability to erect their penis, i.e., some men might require ED drugs to compensate for their lower physical activity. However, to account for this, we adjusted for the number of sex partners since their previous visit, using a measure of frequency of self-reported sexual intercourse, and medical conditions, such as history of congestive heart failure, stroke, bladder and prostate surgery, as well as HCV infection. Third, sexual partner HIV serostatus and use of pre-exposure prophylaxis (PrEP) may have an impact on the outcome; i.e., immune cells and inflammatory markers. However, data were not included in the analyses, since they were not available for most of the study period. Although PrEP was used by $<20\%$ of the men at any time during follow up, the availability of PrEP data occurred at a later time period in our study. The influence of treatment was not assessed since it was beyond the scope of our study, however, we did adjust for viral load and ART used at visit in our outcome models. Finally, our analyses used marginal structural models; thus, we made causal assumptions that there were no uncontrolled confounding, selection bias, measurement error, model misspecification, or violation of positivity whereby ED drug use would have been deterministically given (Hernan et al., 2000; Hernan & Robins, 2020).

Despite the aforementioned limitations, our study has notable strengths. To our knowledge, this is the first study that has examined the prospective immunologic and inflammatory effects of ED drug use over time among MSMs. Most of the prior evidence has been from animal studies, and there has been a lack of studies evaluating the prospective use of ED drugs in humans and MSMs. Another strength of this study included using a well-studied, large cohort of MSM in the

U.S. This study design allowed for the examination of the effects of ED drug use on immune cells and markers over a 10-year time period, and included socially sensitive data collected through a standardized method. In addition, both MWH and MWOH were included in the analyses. Finally, marginal structural models produced estimates (i.e., mean differences) with efficient standard errors and was able to deal with time-varying exposure and confounding (Daniel et al., 2013; Wang et al., 2017).

Conclusion

We believe our findings contribute to a growing body of evidence demonstrating the positive immunomodulatory effects of ED drugs, and that long-term use of ED drugs is not associated with detrimental effects on immune function among MSM. Future studies should investigate the impact of various ED drugs on diverse subtypes of T-cells, other immune cells and immune modulators. Those studies would provide further understanding of the effects of ED drug use on immune function.

Acknowledgement

The authors thank the investigators, the staff, and the participants of the MACS study for their valuable contributions.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding

This work was supported by Baltimore CRS, U019-HL146201-01; Chicago-Northwestern CRS, U01-HL146240-01; Los Angeles CRS, U01-HL146333-01; Pittsburgh CRS, U01-HL146208-01; Data Analysis and Coordination Center, U01-HL146193-01. The MACS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute of Mental Health (NIMH). Targeted supplemental funding for specific projects was also provided by the National Heart, Lung, and Blood Institute (NHLBI), and the National Institute on Deafness and Communication Disorders (NIDCD). MACS data collection is also supported by UL1-TR001079 (JHU ICTR) from the National Center for Advancing Translational Sciences (NCATS), a component of the National Institutes of Health, and NIH Roadmap for Medical Research. O.A.A. was supported by the National Center for Advancing Translational Science (grant UL1-TR001881) and the National Institute of Biomedical Imaging and Bioengineering (grant R01-EB027650). O.A.A. also benefited from facilities and resources provided by the California Center for Population Research at University of California, Los Angeles, which receives core support (grant R24-HD041022) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Additional support for E.C.S. was provided by the Johns Hopkins University Center for AIDS Research (P30-AI094189).

References

Aversa, A., Vitale, C., Volterrani, M., Fabbri, A., Spera, G., Fini, M., & Rosano, G. M. (2008). Chronic administration of Sildenafil improves markers of endothelial function in men with type 2 diabetes. *Diabetic Medicine*, *25*(1), 37–44.

Barnes, H., Brown, Z., Burns, A., & Williams, T. (2019). Phosphodiesterase inhibitors for pulmonary hypertension. *The Cochrane Database of Systematic Reviews*, *1*(10), CD012621.

Barnett, C. F., & Machado, R. F. (2006). Sildenafil in the treatment of pulmonary hypertension. *Vascular Health and Risk Management*, *2*(4), 411–422.

Califano, J. A., Khan, Z., Noonan, K. A., Rudraraju, L., Zhang, Z., Wang, H., Goodman, S., Gourin, C. G., Ha, P. K., Fakhry, C., Saunders, J., Levine, M., Tang, M., Neuner, G., Richmon, J. D., Blanco, R., Agrawal, N., Koch, W. M., Marur, S., … Borrello, I. (2015). Tadalafil augments tumor specific immunity in patients with head and neck squamous cell carcinoma. *Clinical Cancer Research*, *21*(1), 30–38. https://doi.org/10.1158/1078-0432.CCR-14-1716

Cao, W., Mehraj, V., Kaufmann, D. E., Li, T., & Routy, J.-P. (2016). Elevation and persistence of CD8 T-cells in HIV infection: The Achilles heel in the ART era. *Journal of the International AIDS Society*, *19*(1), 20697. https://doi.org/10.7448/IAS.19.1.20697

Chrysant, S. G. (2013). Effectiveness and safety of phosphodiesterase 5 inhibitors in patients with cardiovascular disease and hypertension. *Current Hypertension Reports*, *15*(5), 475–483. https://doi.org/10.1007/s11906-013-0377-9

Daniel, R. M., Cousens, S. N., De Stavola, B. L., Kenward, M. G., & Sterne, J. A. (2013). Methods for dealing with time-dependent confounding. *Statistics in Medicine*, *32*(9), 1584–1618.

Deeks, S. G. (2011). HIV infection, inflammation, immunosenescence, and aging. *Annual Review of Medicine*,

62, 141–155. https://doi.org/10.1146/annurev-med-042909-093756

Detels, R., Phair, J. P., Saah, A. J., Rinaldo, C. R., Murioz, A., Kaslow, R. A., Seminara, D., Schrager, L., & Vermund, S. (1992). Recent scientific contributions to understanding HIV/AIDS from the Multicenter AIDS Cohort Study. *Journal of Epidemiology*, *2*(2sup), 11–19. https://doi.org/10.2188/jea.2.2sup_11

Fisher, D. G., Malow, R., Rosenberg, R., Reynolds, G. L., Farrell, N., & Jaffe, A. (2006). Recreational Viagra use and sexual risk among drug abusing men. *American Journal of Infectious Diseases*, *2*(2), 107–114.

Harte, C. B., & Meston, C. M. (2011). Recreational use of erectile dysfunction medications in undergraduate men in the United States: Characteristics and associated risk factors. *Archives of Sexual Behavior*, *40*(3), 597–606. https://doi.org/10.1007/s10508-010-9619-y

Hernán, M. A., Brumback, B., & Robins, J. M. (2001). Marginal structural models to estimate the joint causal effect of nonrandomized treatments. *Journal of the American Statistical Association*, *96*(454), 440–448. https://doi.org/10.1198/016214501753168154

Hernan, M. A., & Robins, J. M. (2020). *Causal inference: what if*. Chapman & Hall/CRC.

Hernan, M. A., Brumback, B., & Robins, J. M. (2000). Marginal structural models to estimate the causal effect of zidovudine on the survival of HIV-positive men. *Epidemiology*, *11*(5), 561–570.

Karakhanova, S., Yang, Y., Link, J., Soltek, S., von Ahn, K., Umansky, V., Werner, J., & Bazhin, A. V. (2013). Gender-specific immunological effects of the phosphodiesterase 5 inhibitor sildenafil in healthy mice. *Molecular Immunology*, *56*(4), 649–659.

Kim, A. A., Kent, C. K., & Klausner, J. D. (2002). Increased risk of HIV and sexually transmitted disease transmission among gay or bisexual men who use Viagra, San Francisco 2000–2001. *AIDS*, *16*(10), 1425–1428.

Kloner, R. A. (2004). Cardiovascular effects of the 3 phosphodiesterase-5 inhibitors approved for the treatment of erectile dysfunction. *Circulation*, *110*(19), 3149–3155. https://doi.org/10.1161/01.CIR.0000146906.42375.D3

Kniotek, M., & Boguska, A. (2017). Sildenafil can affect innate and adaptive immune system in both experimental animals and patients. *Journal of Immunology Research*, *2017*, 4541958. https://doi.org/10.1155/2017/4541958

McKay, H. S., Bream, J. H., Margolick, J. B., Martínez-Maza, O., Phair, J. P., Rinaldo, C. R., Abraham, A. G., & Jacobson, L. P. (2016). Host factors associated with serologic inflammatory markers assessed using multiplex assays. *Cytokine*, *85*, 71–79. https://doi.org/10.1016/j.cyto.2016.05.016

Meyer, C., Sevko, A., Ramacher, M., Bazhin, A. V., Falk, C. S., Osen, W., Borrello, I., Kato, M., Schadendorf, D., Baniyash, M., & Umansky, V. (2011). Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model. *Proceedings of the National Academy of Sciences*, *108*(41), 17111–17116. https://doi.org/10.1073/pnas.1108121108

Nixon, D. E., & Landay, A. L. (2010). Biomarkers of immune dysfunction in HIV. *Current Opinions of HIV AIDS*, *5*(6), 498–503.

Nunes, A. K., Raposo, C., Luna, R. L., Araújo, S. M., da Cruz-Höfling, M. A., & Peixoto, C. A. (2012). Sildenafil (Viagra(R)) down regulates cytokines and prevents demyelination in a cuprizone-induced MS mouse model. *Cytokine*, *60*(2), 540–551. https://doi.org/10.1016/j.cyto.2012.06.011

Nunes, A. K., Raposo, C., Rocha, S. W. S., Sousa Barbosa, K. P., Almeida Luna, R. L., Cruz-Höfling, M. A., & Peixoto, C. A. (2015). Involvement of AMPK, IKbetaalpha-NFkappaB and eNOS in the sildenafil anti-inflammatory mechanism in a demyelination model. *Brain Research*, *1627*, 119–133. https://doi.org/10.1016/j.brainres.2015.09.008

Pifarre, P., Gutierrez-Mecinas, M., Prado, J., Usero, L., Roura-Mir, C., Giralt, M., Hidalgo, J., & García, A. (2014). Phosphodiesterase 5 inhibition at disease onset prevents experimental autoimmune encephalomyelitis progression through immunoregulatory and neuroprotective actions. *Experimental Neurology*, *251*, 58–71. https://doi.org/10.1016/j.expneurol.2013.10.021

Robins, J. (1986). A new approach to causal inference in mortality studies with a sustained exposure period—Application to control of the healthy worker survivor effect. *Math Modelling*, *7*(9–12), 1393–1512. https://doi.org/10.1016/0270-0255(86)90088-6

Robins, J. M., Hernan, M. A., & Brumback, B. (2000). Marginal structural models and causal inference in epidemiology. *Epidemiology*, *11*(5), 550–560. https://doi.org/10.1097/00001648-200009000-00011

Santi, D., Giannetta, E., Isidori, A. M., Vitale, C., Aversa, A., & Simoni, M. (2015). Therapy of endocrine disease. Effects of chronic use of phosphodiesterase inhibitors on endothelial markers in type 2 diabetes mellitus: a meta-analysis. *European Journal of Endocrinology*, *172*(3), R103–R114. https://doi.org/10.1530/EJE-14-0700

Serafini, P., Meckel, K., Kelso, M., Noonan, K., Califano, J., Koch, W., Dolcetti, L., Bronte, V., & Borrello, I. (2006). Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function. *The Journal of Experimental Medicine*, *203*(12), 2691–2702. https://doi.org/10.1084/jem.20061104

Siewe, B., & Landay, A. (2018). Cellular and soluble immune activation markers in HIV-infected subjects. In T. J. Hope, D. D. Richman, & M. Stevenson (Eds.), *Encyclopedia of AIDS* (pp. 241–248). Springer.

Szczypka, M., & Obmińska-Mrukowicz, B. (2010). Modulating effects of nonselective and selective phosphodiesterase inhibitors on lymphocyte subsets and humoral immune response in mice. *Pharmacological Reports*, *62*(6), 1148–1158. https://doi.org/10.1016/S1734-1140(10)70377-7

Szczypka, M., Ploch, S., & Obmińska-Mrukowicz, B. (2012). Modulation of Th1/Th2 cytokine production by selective and nonselective phosphodiesterase inhibitors administered to mice. *Pharmacological Reports*, *64*(1), 179–184. https://doi.org/10.1016/S1734-1140(12)70744-2

Wada, N. I., Bream, J. H., Martinez-Maza, O., Macatangay, B., Galvin, S. R., Margolick, J. B., & Jacobson, L. P. (2016). Inflammatory biomarkers and mortality risk among HIV-suppressed men: A multisite prospective cohort study. *Clinical Infectious Diseases*, *63*(7), 984–990.

Wada, N. I., Jacobson, L. P., Margolick, J. B., Breen, E. C., Macatangay, B., Penugonda, S., Martínez-Maza, O., & Bream, J. H. (2015). The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. *AIDS*, *29*(4), 463–471. https://doi.org/10.1097/QAD.0000000000000545

Wang, A., Nianogo, R. A., & Arah, O. A. (2017). G-computation of average treatment effects on the treated and the untreated. *BMC Medical Research Methodology*, *17*(1), 3.

Weed, D. T., Vella, J. L., Reis, I. M., De la Fuente, A. C., Gomez, C., Sargi, Z., Nazarian, R., Califano, J., Borrello, I., & Serafini, P. (2015). Tadalafil reduces myeloid-derived suppressor cells and regulatory T cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma. *Clinical Cancer Research*, *21*(1), 39–48. https://doi.org/10.1158/1078-0432.CCR-14-1711

Yildirim, A., Ersoy, Y., Ercan, F., Atukeren, P., Gumustas, K., Uslu, U., & Alican, I. (2010). Phosphodiesterase-5 inhibition by sildenafil citrate in a rat model of bleomycin-induced lung fibrosis. *Pulmonary Pharmacology & Therapeutics*, *23*(3), 215–221. https://doi.org/10.1016/j.pupt.2009.11.002

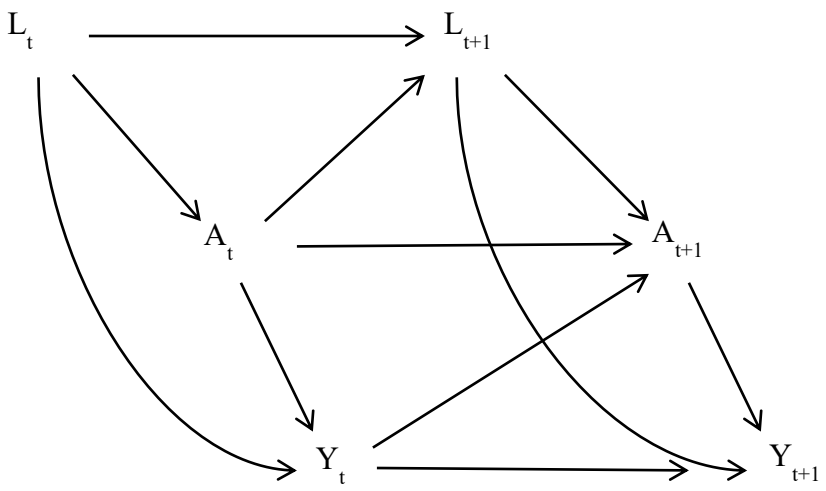
APPENDIX: SUPPLEMENTARY MATERIALS*

* Additional information on variables included in all outcome models of the g-computation for the estimation of mean differences in ED drug users and non-users can be obtained by contacting the author.

Supplemental Table 1. Average number visits and days since last visit among study participants

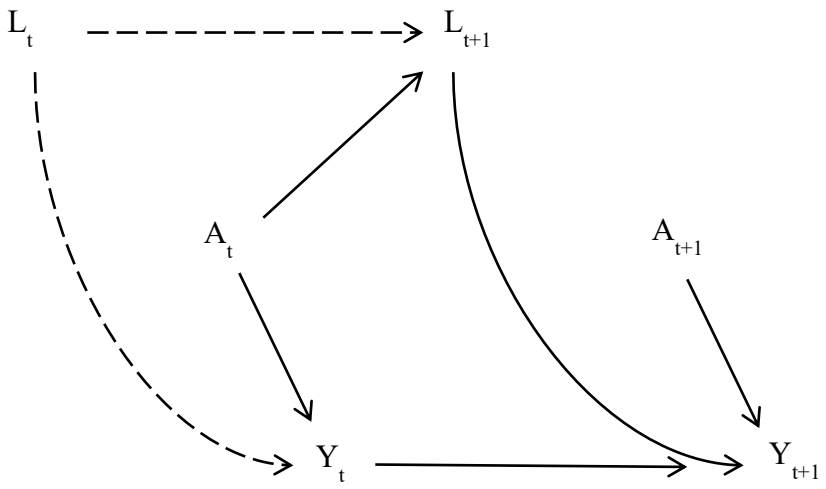
	Number of visits			Days between visits		
	N	Mean	SD	N*	Mean	SD
Total†	1,636	22.1	10.8	36,095	201.8	156.7
MWH	1,391	21.1	11.3	29,343	203.6	166.2
MWOH	307	22.0	9.7	6,752	191.8	107.2

* Total number of observations in the study.
† MWH and MWOH do not add up to the total number since some MWOH seroconverted during the study period, contributing to both groups.



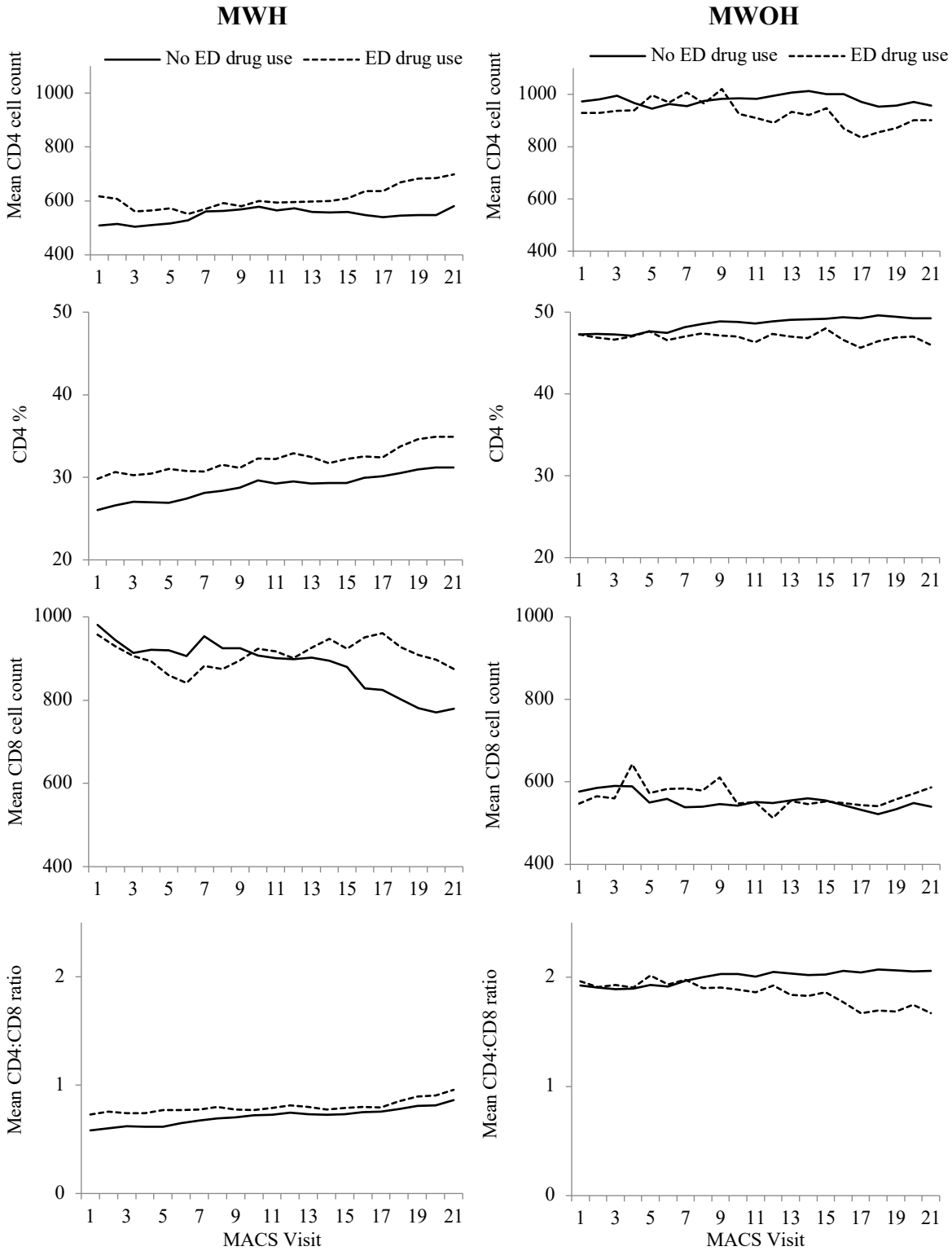
Supplemental Figure 1. Simplified directed acyclic graph (DAG) showing the relationships between exposure (A), outcome (Y) and confounding (L) variables over time (t)

Exposure (A) is the ED drug use and outcome (Y) is CD4 or CD8 cell outcomes, or immune biomarkers. Time-varying confounding variable (L) is the set of variables listed in Appendix Tables 1 and 2. Time, *t*, is the study visit for both MWH and MWOH in the MACS. The variable set L_{t+1} is a causal intermediate between A_t to Y_{t+1} (where it should not be adjusted for) but confounds the effect of A_{t+1} on Y_{t+1} (where it should be adjusted for).



Supplemental Figure 2. Simplified directed acyclic graph (DAG) after intervention on the exposure (A) at each time point (t)

Time, *t*, is the study visits for both MWH and MWOH. Exposure, A, is the assigned ED drug use after intervention and outcome, Y, is the potential outcome. 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. Each A is the exposure intervention. Consequences of any A (that is, variables L and Y at the end of the arrows emanating from any A) now represent potential outcomes. Solid arrows depict the causal pathways of interest.

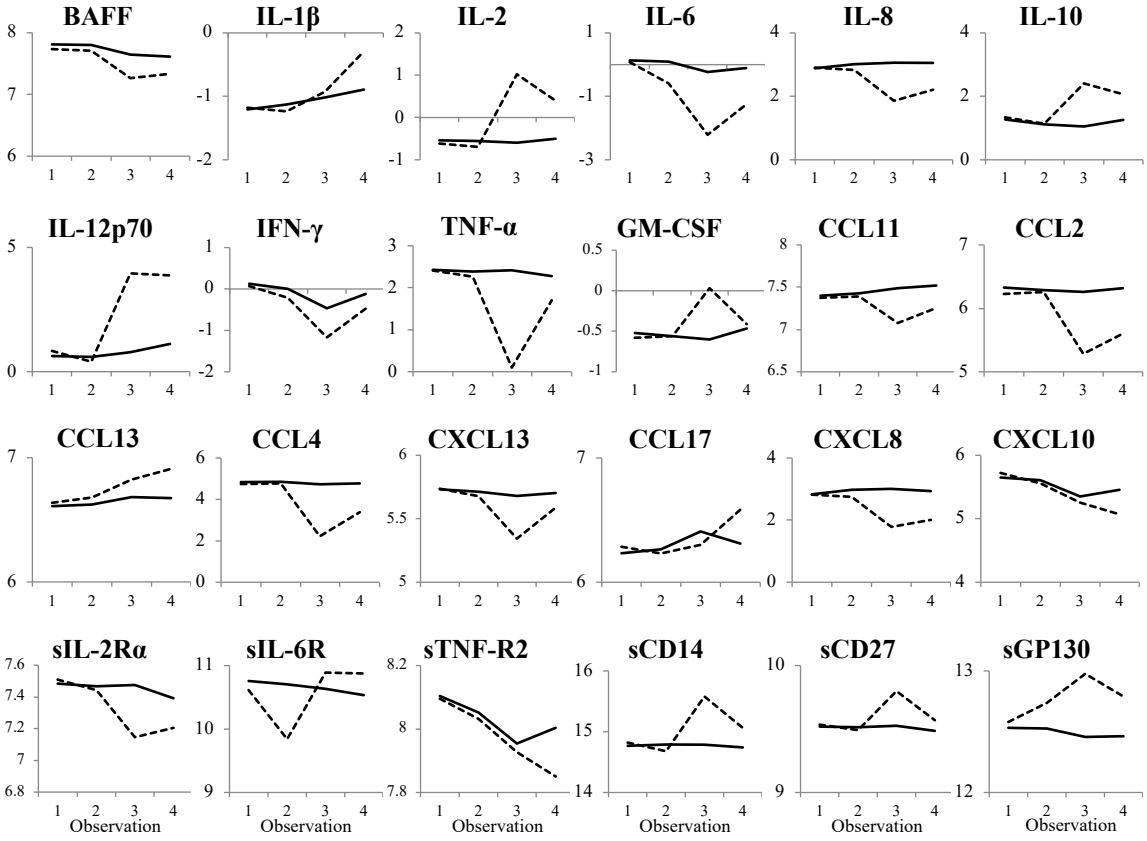


Supplemental Figure 3. Mean CD4 cell counts, CD4%, CD8 cell counts and CD4:CD8 ratio over time comparing ED drug users and non-users among MWH and MWOH participants

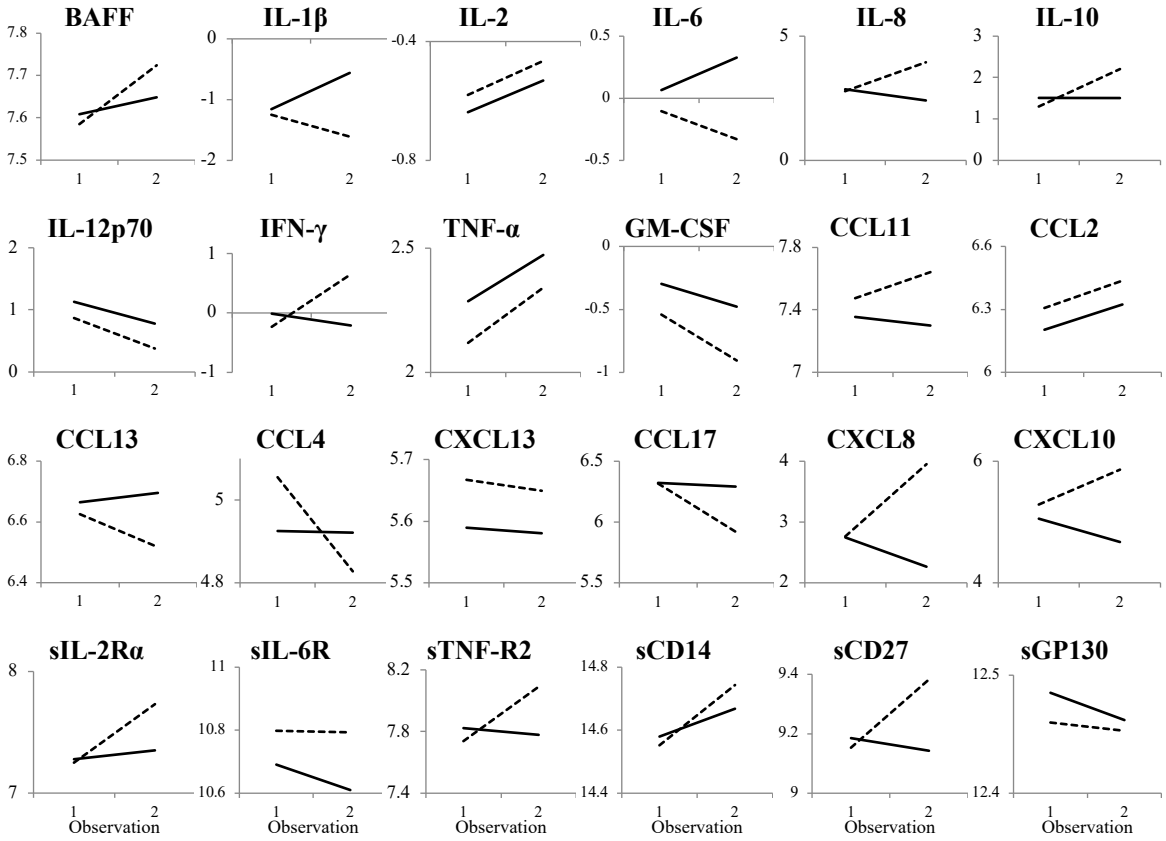
MWH is shown on the left and MWOH is on the right column. Horizontal axis represents MACS study visits (1-21), where visit 1 is the baseline and visit 21 represents 10 years of follow-up. Dashed lines

represent ED drug users and solid lines show non-users. Mean levels are computed from the potential outcomes using g-computation and variables shown in Appendix Table 1.

a. Mean levels of immune biomarkers over time comparing ED drug users and non-users among MWH participants



b. Mean levels of immune biomarkers over time comparing ED drug users and non-users among MWOH participants



Supplemental Figure 4:

a. Mean levels of immune biomarkers over time comparing ED drug users and non-users among MWH participants

Horizontal axis shows the ARRA1 study observations, where observation 1 is the baseline. Vertical axis represents log-transformed outcomes. Dashed lines represent ED drug users and solid lines show non-users. Mean levels are computed by potential outcomes from g-computation using variables in Appendix Table 2.

b. Mean levels of immune biomarkers over time comparing ED drug users and non-users among MWOH participants

Horizontal axis shows the ARRA1 study observations. Vertical axis represents log-transformed outcomes. Dashed lines represent ED drug users and solid lines show non-users. Mean levels are computed by potential outcomes from g-computation using variables in Appendix Table 2.