

**The Association of Sexual Behavior with Systemic Inflammation and Increased HIV-1
Susceptibility in MSM**

by

Grace N. Abror

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This thesis was presented

by

Grace N. Abror

It was defended on

December 10, 2021

and approved by

Committee Chair

Yue Chen, Ph.D. - Associate Professor
Infectious Disease and Microbiology
Graduate School of Public Health
University of Pittsburgh

Committee Member

Sarah Krier, Ph.D. - Assistant Professor
Infectious Disease and Microbiology
Graduate School of Public Health
University of Pittsburgh

Committee Member

Charles R. Rinaldo, PhD - Professor
Pathology, Infectious Disease and Microbiology
School of Medicine, Graduate School of Public Health
University of Pittsburgh

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Grace N. Abror, MPH

University of Pittsburgh, 2021

Background: HIV/AIDS has disproportionately affected MSM since the beginning of the HIV/AIDS epidemic. Sexual behaviors, such as receptive anal intercourse, are thought to be a major contributor to this increased risk for HIV-1 acquisition among MSM. Although it has been found that inflammation in the rectal mucosa plays a significant role in this relationship, little has been discovered about the role systemic inflammation may play. Therefore, this study aims to evaluate which sexual behavior is most significantly associated with HIV-1 seroconversion in a group of MSM from the Multicenter Aids Cohort Study (MACS) and whether that sexual behavior is associated with systemic inflammation.

Methods: In this study, we analyze 1984/1985 sexual activity data, CD4⁺/CD8⁺ ratio data and plasma inflammatory cytokine data obtained from 109 HIV-1 seroconverters (SC) and 156 HIV-1 negative controls (NC) from the MACS, prior to and post- HIV-1 seroconversion.

Results: Receptive anal intercourse was found to be the sexual behavior most significantly associated with HIV-1 seroconversion, prior to and post-HIV-1 seroconversion. Overall, no significant association was found between receptive anal intercourse and systemic inflammation. However, prior to HIV-1 seroconversion, IP10 ($p = <0.01$), sCD163 ($p = 0.50$), and CRP ($p = 0.40$) levels had a tendency to increase with increasing numbers of receptive anal intercourse partners.

Conclusion: The results of this study provide evidence to support that receptive anal intercourse is the sexual behavior most significantly associated with HIV-1 seroconversion in MSM. In addition, when evaluating the association between the number of receptive anal intercourse and plasma inflammatory response markers prior to and post- HIV-1 seroconversion, it was found that, overall, there was no significant association.

Public Health Significance: In order to discover novel treatments for HIV, it is necessary to explore factors that may increase MSMs' susceptibility to HIV-1 infection and characterize the pathway by which they do so.

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Preface

I would like to thank my thesis advisor and committee chair, Dr. Yue Chen, for first and foremost reaching out to me inviting me to do this study as a follow-up to her recently published study. I would also like to thank her for her all the time, support, and guidance she provided me with to be able to finish my thesis in this short amount of time. She assisted me immensely in developing this study as well as this paper.

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Finally, I would like to thank Drs. Peddada and Lin, of the NIH, who took time out of their busy schedules to provide expertise and guidance regarding the statistical analyses done throughout this study. This study may have not been possible without them!

List of Abbreviations

- MSM** Men who have sex with men
- HIV** Human Immunodeficiency Virus
- AIDS** Acquired Immune Deficiency Syndrome
- HTLV-II** Human T-lymphotropic virus 2
- MACS** Multicenter Aids Cohort Study
- STD** Sexually transmitted disease
- STI** Sexually transmitted infection
- HSV II** Herpes Simplex Virus 2
- PCP** Pneumocystis carinii pneumonia
- KS** Kaposi's sarcoma
- SC** HIV-1 seroconverter
- NC** HIV-1 negative control

1.0 Introduction

Since the beginning of the HIV/AIDS epidemic in the United States, men who have sex with men (MSM) have been disproportionately affected by HIV-1. Although MSM account for only 2% of the US population, they accounted for 69% of new HIV infections in 2019 and account for 55% of the American population currently living with HIV ("HIV testing and risk behaviors among gay, bisexual, and other men who have sex with men - United States," 2013). Sexual behavior, specifically anal intercourse, among MSM has been identified to be the route of exposure most attributed to the high incidence rates of HIV-1 infection in this group (Detels et al., 1989). Studies have reported that receptive anal intercourse is the greatest risk factor in contracting HIV-1 among MSM (Kingsley et al., 1987) (Detels et al., 1989) (McDaid & Hart, 2010). Anal intercourse can result in damage to the rectal mucosa, STDs, and rectal mucosa inflammation, all of which increase the risk of HIV-1 acquisition (Kelley et al., 2017). Inflammation is a major contributor to HIV-1 susceptibility in MSM (Morgan et al., 2019) (Pebody, 2019) (Arnold et al., 2016). Currently, it is suggested that inflammation in the rectal mucosa can result in a systemic inflammatory response through the concept of leaky gut and bacterial translocation (Brenchley et al., 2006). This finding helps to characterize a pathway between sexual intercourse in MSM and systemic inflammation. The current study aimed to evaluate which sexual behavior is most significantly associated with HIV-1 seroconversion in a group of MSM from the Multicenter Aids Cohort Study (MACS) as well as evaluate the relationship of the sexual behavior and systemic inflammation in that group.

1.1 Early HIV/AIDS Epidemic and MSM

In the summer of 1981, a spike in cases of *Pneumocystis carinii* pneumonia (PCP) and Kaposi's sarcoma (KS), occurred among young, otherwise healthy, gay men in the U.S. marked the beginning of what would later be known as the HIV/AIDS epidemic. Seeing these diseases in healthy individuals who all happened to be gay men sparked alarm among health officials across the country. From the beginning, it was speculated that the problem was most likely due to a sexually transmitted infectious agent. Later in 1981 when heterosexual drug users began to present with the same diseases, it was suggested that the unknown infectious agent could also be transmitted through blood ("HIV testing and risk behaviors among gay, bisexual, and other men who have sex with men - the United States," 2013).

Because these unusual occurrences of PCP and KS were disproportionately affecting gay men, PCP and KS were labeled as "Gay Men's Pneumonia" and "Gay Cancer", respectively, and the immune suppression that caused these diseases was labeled as "Gay-Related Immune Deficiency (GRID)." It wasn't until over a year after AIDS first emerged that the Centers for Disease Control (CDC) coined the term, "Acquired Immune Deficiency Syndrome (AIDS)". The association of AIDS with gay men in the early epidemic resulted in severe social stigma surrounding MSM, an already stigmatized subset of the population. In the early stages of the pandemic, very little money was allocated to researching AIDS. For this reason, it wasn't until 1984, 3 years after AIDS first emerged, that the retrovirus that caused AIDS, first known as HTLV-II and later named HIV, was discovered (AIDS Crisis Timeline, 2021).

One of the first major studies funded to study AIDS in gay and bisexual MSM was the Multicenter Aids Cohort Study (<https://statepi.jhsph.edu/mwccs/>). The MACS is a prospective cohort study of HIV-1 infection in MSM established in 1983 at 4 sites (Baltimore, Maryland/Washington, DC; Chicago, Illinois; Los Angeles, California; Pittsburgh, Pennsylvania). In 1984, 4954 gay and bisexual MSM were enrolled in the study, and enrollment has been ongoing since. MACS participants have been studied at semiannual clinic visits with standardized interviews, physical examinations, and phlebotomy for laboratory testing, with storage of plasma and serum and viable peripheral blood mononuclear cells (Kaslow et al., 1987). Early contributions of the MACS to HIV/AIDS included research on seroconversion risk, T cell decline, progression to AIDS, and host genetics. The MACS and similar early studies provide the foundation for much of the knowledge on HIV/AIDS research we have today.

1.2 Sexual Behaviors in MSM and Increased Susceptibility to HIV-1 Infection

Since the beginning of the HIV/AIDS epidemic, major strides have been made in identifying routes of transmission of HIV-1. Routes of HIV transmission identified include sharing needles or other types of drug injection equipment, perinatal transmission, and sexual contact. An essential area of research over the past few decades has been characterizing the risk of transmission for each of these routes and identifying the populations that may be at the highest risk of contracting HIV through these routes. A 2014 meta-analysis reported estimated “per-act probability of acquiring HIV from an infected source” for several different exposure routes. These estimates were reported as risk per 10,000 exposures to an infected source. The findings showed that blood transfusions posed the greatest risk per 10,000 exposures to an infected source at 9250

per 10,000 exposures, followed by mother-to-child transmission (2260 per 10,000 exposures) and receptive anal intercourse (138 per 10,000 exposures) (Patel et al., 2014).

It has been established that a major contributor to disproportionately high HIV/AIDS infection rates among MSM is risky sexual behavior, specifically anal sex (McDaid et al). Kingsley et al reported that receptive anal intercourse accounted for nearly all of the new HIV infections among the MSM enrolled in the MACS study and therefore suggested that receptive anal intercourse is the riskiest behavior in contracting HIV-1 (Kingsley et al). This finding has been confirmed many times since 1987 (Pebody, 2019). When compared to the per act probability of acquiring HIV for condomless receptive vaginal sex (8 per 10,000 exposures), the per act probability for acquiring HIV is over 17 times greater for condomless receptive anal intercourse (138 per 10,000 exposures) (Patel et al., 2014)

One reason receptive anal intercourse is found to be the riskiest sexual behavior for contracting HIV-1 is that the lining of the rectum is very thin and therefore prone to tears during intercourse. Tears in the rectal lining can allow HIV-1 to enter the bloodstream directly (Kelley et al., 2017). Not only can the compromise of the rectal lining allow for direct HIV-1 entry into the bloodstream, but it can also allow for the entry of other STIs pathogens which are known to increase an individual's risk for contracting HIV-1. Some STDs/STIs cause ulcers, or lesions that provide a direct route of entry of HIV-1, similar to a tear in the rectal lining. Furthermore, STDs/STIs often result in an increased number of HIV-infection-prone immune cells, such as activated T-cells and dendritic cells, at the site of infection which gives HIV a target cell to infect in the genitalia (Pebody, 2019). One study found that HSV-2 increased the risk of acquiring HIV-1 by 170% among MSM (Looker et al., 2017). Insertive anal intercourse, although not found to be as risky as receptive anal intercourse, can also be a risk factor for contracting HIV-1 among MSM.

A 2004 study discovered that rectal secretions in HIV+ MSM contain more HIV than their blood or semen. Therefore, during insertive anal intercourse, HIV can enter the bloodstream of the insertive partner through the urethral meatus at the tip of the penis, or through tears on the penis (Zuckerman et al., 2004).

1.3 Inflammatory Response and HIV

Inflammation can be both, directly and indirectly, related to HIV-1 acquisition in MSM. Rectal mucosa inflammation is a well-known source of increased HIV-1 susceptibility in MSM. Rectal mucosa inflammation in MSM most commonly results from damage to the rectal mucosa and STDs/STIs (Pebody, 2019). A 2016 study found that condomless receptive anal intercourse results in greater CD8+ T-cell proliferation and production of pro-inflammatory cytokines in the rectal environment (Kelley et al., 2017). HIV-1 can directly infect the immune cells in rectal mucosa which are activated as a result of damage to the rectal mucosa or active infection (McKinnon & Kaul, 2012). Not only has rectal mucosa inflammation been associated with an increased risk for HIV-1 infection, plasma inflammatory immune response factors, such as pro-inflammatory cytokines, have also been found to increase the risk for contracting HIV-1 in MSM (Morgan et al., 2019).

Acute systemic inflammation is characterized primarily by immune cell activation and elevated levels of pro-inflammatory biomarkers (Chen et al., 2018). While inflammation and immune activation are essential to the initiation of a protective host immune response, some pathogens, such as HIV, take advantage of this inflammation and immune activation (Arnold et al., 2016). Because HIV attacks immune cells, inflammation provides HIV-1 with an increased

number of target cells to infect. Thus causing inflammation to increase an individual's susceptibility to HIV-1 infection. One study found that genital levels of alpha-defensins, which are antimicrobial peptides that induce proinflammatory cytokine release by T cells, were associated with increased rates of HIV acquisition in men (Levinson et al., 2009).

A 2006 study found significantly increased levels of circulating microbial products (lipopolysaccharide), speculated to have originated in the gastrointestinal tract, in chronically HIV-infected individuals. Lipopolysaccharide was used as a marker for microbial translocation which therefore suggests that local inflammation can lead to microbial translocation which can lead to systemic inflammation. This finding provides evidence for a pathway between local inflammation and systemic inflammation. This pathway is applicable in connecting inflammation in the rectal mucosa, caused by sexual behaviors, to systemic inflammation which leads to increased HIV-1 susceptibility (Brenchley et al., 2006).

As previously mentioned, the comprehensive data and biological samples collected from MACS participants in the early HIV epidemic are invaluable for studying HIV/AIDS prior to ART. One recent study that used the data and biological samples collected from MACS participants in 1984-1985 was performed by Chen et al (2021) This study examined the association between the gut microbiome, inflammatory response, and HIV seroconversion in MSM. 205 study participants were divided into two groups, HIV seroconverters (SC) and HIV negative controls (NC). Inflammatory markers, gut microbiome, and fecal short-chain fatty acids from these individuals were analyzed prior to seroconversion (visit 1) and post-seroconversion (visit 2). The study found that levels of inflammatory biomarkers were significantly higher among SC prior to seroconversion compared to NC. These findings also found that both gut microbiome and systemic inflammation were associated with HIV-1 seroconversion (Chen et al., 2021).

1.4 Research Question

Our previous study found there are significant changes both in plasma inflammatory cytokine levels and gut microbiome composition in the SC group before HIV infection compared to NC. However, it is unclear whether there is a relationship between certain sexual behavior, plasma cytokine levels, and HIV seroconversion in the MACS study participants. Therefore, this study aims to explore whether there is an association between sexual behavior in MSM, the inflammatory response, and HIV-1 infection. This study is one of the few which explore this association in participants who are not taking ART drugs, which could influence the pathway.

The objective of this thesis is to explore the following questions.

1. Which sexual behavior is most significantly associated with HIV-1 seroconversion in a group of MSM from MACS?
2. Is that sexual behavior associated with systemic inflammation within this sample of MACS MSM?

2.0 Methods

2.1 Study Participants and Plasma Cytokine Measurements

During the early phase of the HIV-1 pandemic (1984-1985), MACS participants were instructed to provide stool, urine, semen and oral wash samples at each clinical research visit, which have been preserved at -80C without additives or preservatives. Enrollment and clinical research of the MACS participants began April 1, 1984, with clinical research visits at 6-month intervals thereafter. During that early period, a number of the MACS participants were infected by HIV-1. HIV-1 seroconversion was determined by enzyme-linked immunosorbent assay (ELISA) and confirmed by Western blot with the participants' serum samples. The HIV-1 infection date was estimated as the midpoint between the last seronegative and first seropositive clinic research visits. In the current study, we examined plasma samples from the clinic research visits flanking the estimated HIV-1 infection time point from 109 HIV-1 seroconverters, and 156 HIV-1 uninfected MSM controls collected during the same time period. The paired samples spanned approximately 6 months, which included an estimated 3 months prior to and 3 months after HIV-1 infection. HIV-1 seroconverters and controls were matched by study centers. All samples were obtained in 1984-1985, prior to the antiretroviral therapy used to treat HIV.

For the heparinized plasma samples, the inflammatory cytokines soluble CD14 (sCD14), soluble scavenger receptor CD163 (sCD163), C-reactive protein (CRP), interferon gamma-induced protein 10 (IP-10), and lipopolysaccharide-binding protein (LBP) were measured with the Luminex xMAP platform (Luminex, Northbrook, IL, USA), according to the manufacturer's instructions. The data were collected and analyzed using a BioPlex 200 apparatus and BioPlex

Manager Software (Bio-Rad, Hercules, CA, USA). In addition, the inflammatory cytokine interleukin 6 (IL-6) was measured in the plasma samples by ELISA using a commercial ELISA kit (R&D, Minneapolis, MN, USA) following the manufacturer's instructions.

2.2 Sexual Activity Data

Sexual activity data reported in this study are based on respondents' answers to MACS interview questions obtained in 1984-1985. The MACS sexual behaviors interviews elicited the number of partners with whom various sexual behaviors were performed in their lifetime prior to visit 1 (baseline), 2 years before visit 1, six months before visit 1, and during the six-month follow-up (Visit 2). In these time-frames, the questions were identical. In this study, the sexual behavior we focus on is male-to-male intercourse including oral-genital intercourse (to ejaculation and not to ejaculation) and anal intercourse (to ejaculation and not to ejaculation). We specifically look at the following: number of male anal intercourse partners a participant has had in their lifetime ("male intercourse ever"), number of male anal intercourse partners a participant had in the 6 months prior to each visit (visit 1 and visit 2), number of partners, in the 6 months prior to each visit, with whom the participant was the receiving partner during anal intercourse ("receptive anal intercourse"), and number of partners, in the 6 months prior to each visit, with whom the participant was the insertive partner during anal intercourse ("insertive anal intercourse"). In this study, the preferred measure of sexual behavior was "number of partners with whom the sexual act was performed" rather than "number of times a sexual act was performed" as the former more accurately depicts the risk-taking behavior of the participant. The greater number of partners by which an individual performs a sexual act, the greater the chance that the sexual behavior was

performed with an HIV+ individual. Condom usage during these sexual activities was not evaluated as we could not assess the accuracy of the data in terms of correct and effective usage of the condoms.

Data for “male intercourse”, “receptive anal intercourse”, and “insertive anal intercourse” are sorted by visit. Data for male intercourse ever was only collected at Visit 1 (baseline). The study was conducted with institutional review board approval from all participating institutions.

2.3 Statistical Analyses

Analysis of sexual behaviors between NC and SC

Violin plots were used to compare the distribution of the number of partners, at each visit, for each sexual behavior evaluated in this study, in SC versus NC. Violin plots are a method of plotting numeric data and can be considered a combination of the box plot with a kernel density plot. There are several components to a violin plot. The outer shading represents a kernel density plot of the data and is mirrored across the centerline. The black bar in the center of the violin represents the interquartile range, and the dot in the middle of that represents the median value of the data. The thinner portion of the centerline, on either side of the thick portion, represents the 95% confidence interval. The number of partners for each sexual activity is presented in log scale to reduce skewness, therefore enhancing visibility of the trend. Because “male intercourse ever” could be associated with age, it was necessary to assure that age was not a confounding variable in the plot. Therefore, a two-sample Kolmogorov-Smirnov test for equality of distribution functions was run with age as the main variable and conversion status as the binary variable. The results of the test revealed that age was not a confounding variable in the plot ($p = 0.13$).

To determine which sexual behavior was most significantly associated with HIV conversion status, a logistic regression model for each visit was run with conversion status as the dependent variable and “receptive anal intercourse”, “insertive anal intercourse” and “age” as the independent variables. “Age” was added to account for the effect of age in the data. To avoid collinearity, “Male intercourse ever” and “male intercourse” were not included as variables in this logistic regression model.

Analysis of CD4⁺/CD8⁺ ratios and inflammatory cytokines difference between groups

To ensure the between-group sample size balance, for each visit, participants were organized into 4 groups based on their number of receptive anal intercourse partners. Group 1 had no (zero) receptive anal intercourse partners, group 2 had one receptive anal intercourse partner, group 3 had two receptive anal intercourse partners, and group 4 had 3 or more receptive anal intercourse partners. The resulting sample size for each group at visit 1 are N = 64 for group 1, N = 58 for group 2, N = 30 for group 3 and N = 92 for group 4 and at visit 2 are N = 40 for group 1, N = 31 for group 2, N = 21 for group 3 and N = 41 for group 4. To evaluate the relationship between receptive anal intercourse and inflammatory response markers, box, and whiskers plots were used to show the distribution of CD4⁺/CD8⁺ ratio and levels of IL6, sCD163, IP10, CRP, LBP, and sCD14 between each group, at each visit. A Kruskal-Wallis test was performed to determine the significance of the difference in levels of inflammatory response markers between the groups, at each visit. These p-values, however, do not convey the significance of the overall trend for each inflammatory response marker, at each visit. The log₂ transformed CD4⁺/CD8⁺ ratio was evaluated alongside log₁₀ transformed pro-inflammatory cytokine levels as a measure of immune status. Although individually CD4⁺ and CD8⁺ are important, it is their relative value that

is biologically more meaningful because these are compositional. For each of the cytokines, analyses were performed using the \log_{10} transformed value.

Although, in this study, the association of receptive anal intercourse and systemic inflammation is explored independent of HIV-1 conversion status, it is important to consider how HIV-1 conversion status could impact this association. Therefore, the analysis was also run using the same groups but stratified by HIV-1 conversion status. The results of this analysis were the same as what we see in Figure 3. Therefore, it was decided that we would continue with the analysis run independent of HIV-1 conversion status as this more directly answers the research question.

3.0 Results

Study participants, clinical samples and sexual behaviors

For this study, the HIV-1 infection events in SC were retrospectively reconfirmed by RT-PCR with cryopreserved plasma, with HIV-1 antibody and RNA being negative at visit 1 and both being positive at visit 2 with approximately six-month intervals. The HIV-1 negativity of NC was confirmed by negative plasma HIV-1 antibody results from both visits. All study participants were self-defined MSM. The SC did not receive ART due to the unavailability of effective antiretroviral drugs in the early years of the HIV-1 pandemic. There were 35 SC (32%) and 77 NC (49%) with plasma samples from both visits 1 and 2, 52 (48%) SC and 79 NC (51%) only with visit 1 samples, 22 SC (20%) and 0 NC (0%) with only visit 2 samples. When looking at the time period between the two visits (days), the median was 188 days for SC, and 182 days for NC ($p < 0.01$) (Table 1). Overall, the NC had a slightly older median age ($p=0.47$) (Table 1). Compared to the NC, at each visit, the SC had a greater median number of partners across each sexual behavior evaluated in this study; “male intercourse ever”, “male intercourse”, “receptive anal intercourse”, and “insertive anal intercourse” (Table 1).

Sexual behaviors among participants by HIV-1 conversion status

As displayed in Figure 1, violin plots were used to compare the distribution of the number of partners, at each visit, for each sexual behavior evaluated in this study, in SC versus NC. When looking at the violin plots for “male intercourse ever” (number of male anal intercourse partners a participant has had in their lifetime), we can see that, while the distribution is mostly normal in both the SC and NC, the median number of partners and interquartile range are greater in the SC compared to the NC (Figure 1A).

The violin plots for “male intercourse” (number of male anal intercourse partners a participant had in the 6 months prior to each visit) show similar results with the SC having a greater median number of partners and interquartile range compared to the NC, at each visit (Figure 1B). The violin plots for “receptive anal intercourse” (number of partners, in the 6 months prior to each visit, with whom the participant was the receiving partner during anal intercourse) also exhibit that at both visits 1 and 2, SC had a greater median number of partners and interquartile range compared to that of NC. The distribution of the data is seen to be normal in SC for both visit 1 and visit 2, and non-normal in NC for both visit 1 and visit 2 (figure 1C). At both visits, the violin plots for “insertive anal intercourse” (number of partners, in the 6 months prior to each visit, with whom the participant was the insertive partner during anal intercourse) also showed a greater median number of partners and interquartile range for SC compared to NC. The distribution of the data for the SC appears to be right-skewed at visit 1 and mostly normal at visit 2 while the distribution of data for the NC appears to be non-normal and right-skewed (Figure 1D). Overall, figure 1 reveals that, compared to NC, SC engaged in risky sexual behaviors with a greater number of partners which indicates their tendency to engage in more risk-taking behaviors.

Association between sexual behavior and conversion status

Once sexual behavior trends were recognized among the NC and SC, the subsequent course of action was to determine which sexual behavior was most significant in predicting HIV seroconversion among the participants examined in this study. Using a logistic regression model with “conversion status” as the dependent variable and “receptive anal intercourse”, “insertive anal intercourse” and “age” as the independent variables, it was discovered that receptive anal intercourse was most significantly associated with HIV-1 seroconversion in this study.

The results of the logistic regression are represented in a forest plot showing the log odds of seroconversion with the 95% confidence interval for each covariate. The line of null effect is found at $x = 0$. Visit 1 forest plot (Figure 2A) indicates that “receptive anal intercourse” was the most significant predictor of HIV seroconversion, with “insertive anal intercourse” being slightly less predictive of HIV seroconversion, and “age” not at all predicting seroconversion. Visit 2 forest plot (Figure 2B) showed similar results, although more extreme. Again, “receptive anal intercourse” had the greatest positive log odds value and was therefore shown to be the most significant predictor of seroconversion. “Insertive anal intercourse” had a very small log odds value and therefore was not shown to be a significant predictor of seroconversion. “Age” also had a negative log odds value showing that it was not a predictor of seroconversion.

Cytokine levels and cell surface marker levels and association with receptive anal partners

After establishing that receptive anal intercourse was the most significant predictor of HIV seroconversion, the next research question to examine was whether increasing numbers of receptive anal intercourse partners was associated with an increased inflammatory response in the participants.

In order to answer the research question, CD4⁺/CD8⁺ ratios as well as plasma inflammatory cytokine levels were evaluated for each group. Groups were established based on number of receptive anal intercourse partners; group 1 was comprised of participants with 0 receptive anal intercourse partners, group 2 had 1 receptive anal intercourse partner, group 3 had 2 receptive anal intercourse partners, and group 4 had 3 or more receptive anal intercourse partners. The plasma inflammatory cytokines evaluated in this study were IL6, sCD163, IP10, CRP, LBP, and sCD14. Figure 3 visually represents the distribution of log₂ transformed values for CD4⁺/CD8⁺ ratios and plasma inflammatory cytokine/biomarker levels for each group, at each visit, in the form

of box plots. At visit 1 (prior to HIV-1 seroconversion), IP10 ($p = <0.01$), sCD163 ($p = 0.50$), and CRP ($p = 0.40$) levels had a tendency to increase with increasing numbers of receptive anal intercourse partners. However, CD4⁺/CD8⁺ ratio ($p = 0.60$), LBP ($p = 0.99$), and IL6 ($p = 0.09$) did not display the same tendency. At visit 2 (post HIV-1 seroconversion), we do not observe any sort of tendency for CD4⁺/CD8⁺ ratio ($p = 0.04$) or IL6 ($p = 0.20$), sCD163 ($p = 0.22$), IP10 ($p = 0.09$), CRP ($p = 0.41$), LBP ($p = 0.55$), and sCD14 ($p = 0.03$) levels to increase with increasing numbers of receptive anal intercourse partners.

Table 1. Participant Characteristics

		HIV Seroconverters (N=109)	HIV Negative Controls (N = 156)	P-value
Data availability		N (%)	N (%)	
Samples from visit 1 & 2 available		35 (32%)	77 (49%)	
Samples from visit 1 available		52 (48%)	79 (51%)	
Samples from visit 2 available		22 (20%)	0 (0%)	
		Median (Range)	Median (Range)	
Time period between samples (days)		188 (141-547)	182 (77-230)	< 0.01
Age at enrollment		35.94 (19-82)	36.94 (21-80)	0.47
Number of male intercourse partners during lifetime		200 (10-999+)	100 (1-999+)	< 0.01
Number of male intercourse partners during previous 6 months	Visit 1	10 (0-500)	5 (0-60)	< 0.01
	Visit 2	7 (0-150)	5 (0-100)	0.03
Number of receptive anal intercourse partners during previous 6 months	Visit 1	3 (0-200)	1 (0-35)	< 0.01
	Visit 2	3 (0-20)	1 (0-10)	< 0.01
Number of insertive anal intercourse partners during previous 6 months	Visit 1	3 (0-300)	1 (0-25)	< 0.01
	Visit 2	2 (0-125)	1 (0-20)	< 0.01

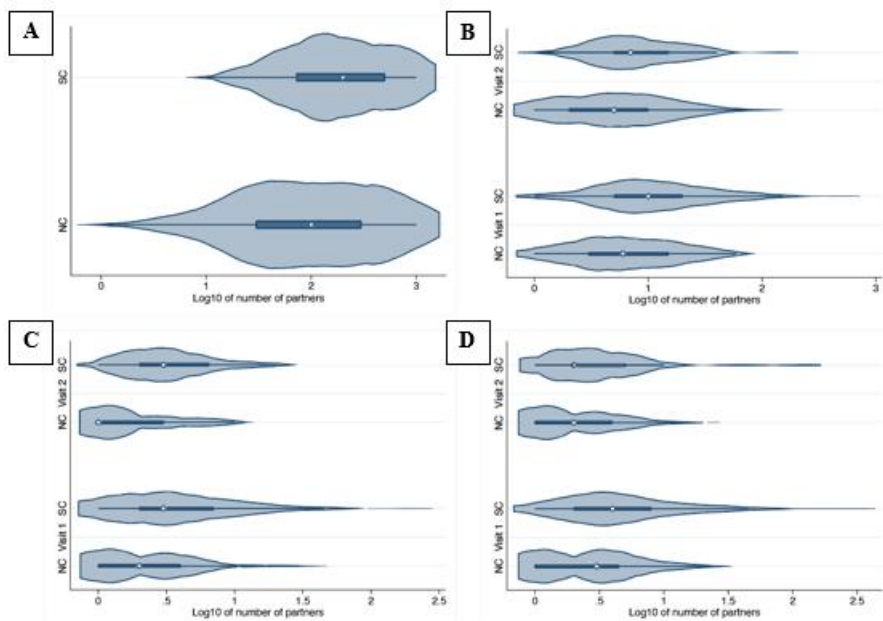


Figure 1. Number of partners with whom a sexual behavior was performed in HIV-1 seroconverters (SC) vs HIV-1 negative controls (NC) prior to (visit 1) and post-seroconversion (visit 2)

(a) Male intercourse ever (b) Male intercourse at visit 1 and visit 2 (c) Receptive anal intercourse at visit 1 and visit 2 (d) Insertive anal intercourse at visit 1 and visit 2

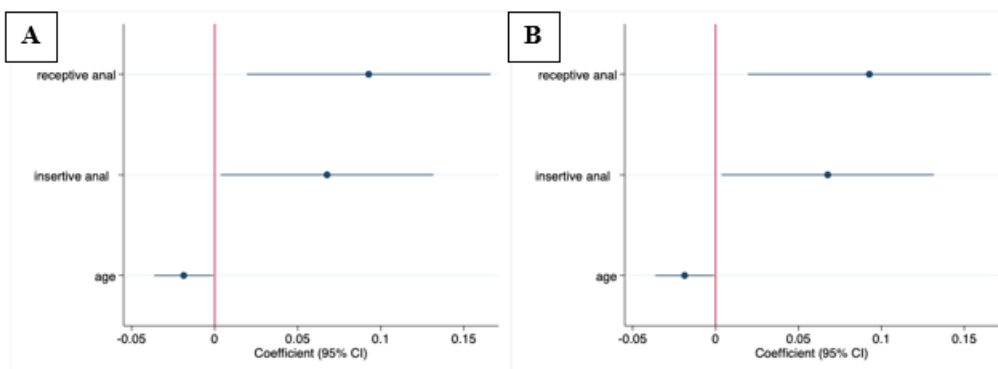


Figure 2. Association of sexual behaviors with HIV-1 seroconversion

(a) Visit 1 (b) Visit 2

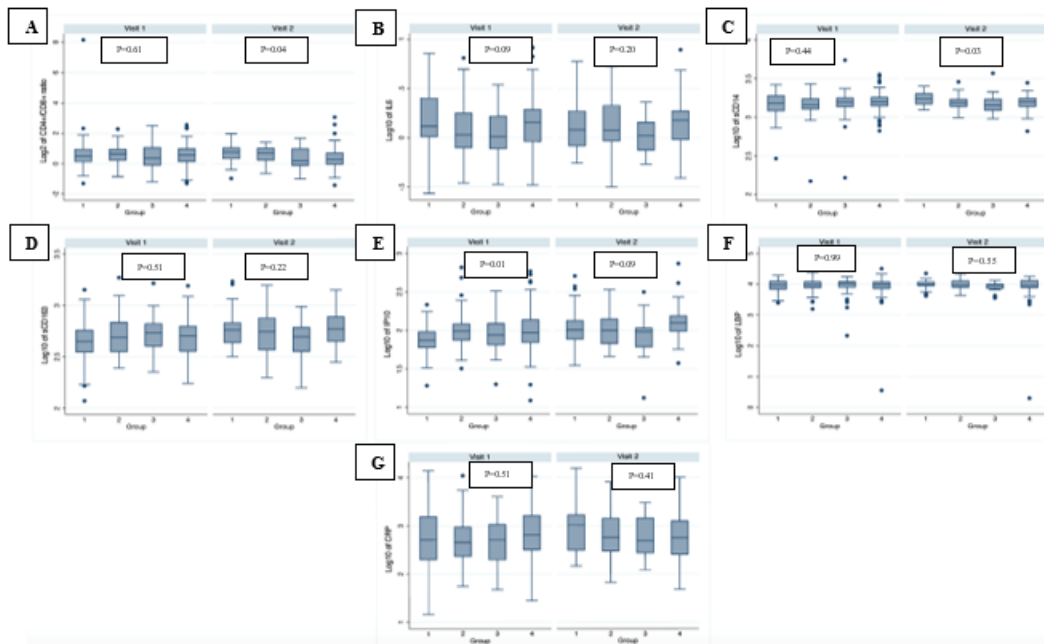


Figure 3. Association of number of receptive anal intercourse with CD4+/CD8+ ratio and cytokine levels

(a) Log₂ of CD4+/CD8+ ratio (b) Log₁₀ of IL6 (c) sCD14 (d) Log₁₀ of sCD163 (e) Log₁₀ of IP10 (f) Log₁₀ of LBP (g) Log₁₀ of CRP. Groups represent the number of receptive anal intercourse partners. Group 1 represents 0 receptive anal intercourse partners group 2 represents 1 receptive anal intercourse partner, group 3 represents 2 receptive anal intercourse partners and group 4 represents 3 or more receptive anal intercourse partners.

4.0 Discussion

The basic premise of this study was to examine which sexual behavior was most significantly associated with receptive anal intercourse and whether that behavior was significantly associated with systemic inflammation and therefore increased susceptibility to HIV-1 seroconversion in a group of MSM from the MACS. To address this, we analyzed sexual activity data, CD4⁺/CD8⁺ ratios, and plasma inflammatory cytokines among HIV-1 seroconverters and HIV-1 negative controls prior to (Visit 1) and post- seroconversion (Visit 2). The results confirmed that receptive anal intercourse was the sexual behavior most strongly associated with HIV-1 seroconversion in this group of MSM (Figure 2), consistent with the literature on HIV risk factors among MSM (Kingsley et al., 1987) (McDaid & Hart, 2010) (Patel et al., 2014). This finding tells us that, compared to those participants with lesser numbers of receptive anal intercourse partners, the participants who had more receptive anal intercourse partners were more likely to have seroconverted between their first and second visit. The rationale for this is as follows, as the number of receptive anal intercourse partners an individual has increases, the chance that one or more of those partners being HIV positive (knowingly or unknowingly) increases as well.

The significant association of receptive anal intercourse with HIV-1 seroconversion may be attributed to increased inflammation of the rectal mucosa among these participants which provides the HIV with an abundance of target cells (McKinnon & Kaul, 2012). One major source of inflammation in the rectal mucosa of MSM is damage to the rectal mucosa. Tears in the rectal mucosa are a common consequence of receptive anal intercourse due to the rectal mucosal lining being thin and delicate (Kelley et al., 2017). These tears in the rectal mucosa result in the influx of immune cells to the area (Arnold et al., 2016). It is also possible that the tears in the rectal mucosa

from receptive anal intercourse created a direct entry route to the bloodstream for HIV-1 during receptive anal intercourse with an HIV+ partner (Pebody, 2019).

Although not examined in this study, another possible cause of increased inflammation in the rectal mucosa of these participants is active STD/STIs. STDs/STIs are more prevalent among MSM (Rice et al., 2016). Active STD/STIs have been found to increase the shedding of HIV particles. Therefore, if a participant was the receptive partner during anal intercourse with an HIV-1 positive individual, who was also infected with an STI, that individual would have had a greater chance of transmitting HIV-1 to the receptive partner during that sexual interaction (Looker et al., 2017) (Houlihan et al., 2012) (Masha et al., 2019). Because this study evaluated participants prior to the availability of ART and PrEP, there was very little protection against HIV-1 for those participants engaging in anal intercourse with an HIV-1 positive individual.

Moreover, in this study, we found that, overall, the number of receptive anal intercourse partners among these MSM was not significantly associated with systemic inflammation (Figure 3). These findings are interesting as there is evidence that receptive anal intercourse is associated with inflammation in the rectal mucosa (Kelley et al., 2017) (McInally et al., 2021) and local inflammation can lead to systemic inflammation through microbial translocation (Brenchley et al., 2006). Because no significant association was found between receptive anal intercourse and systemic inflammation in these MSM, we are led to believe that there is some factor overshadowing the pathway between local inflammation and systemic inflammation. One such factor that has been somewhat explored is the gut microbiome. Chen et al provided evidence for the gut microbiome being a mediator of systemic inflammation and therefore an important factor in HIV-1 seroconversion (2021). It has been established that MSM have a distinct microbiome compared to MSW, independent of HIV status (Noguera-Julian et al., 2016). Therefore, it is

possible that abnormalities in the gut microbiome were directly related to receptive anal intercourse and overshadowed any type of direct relationship between receptive anal intercourse and systemic inflammation.

4.1 Limitations

There are several limitations to this study. Responses from 1984/1985 sexual behavior questionnaires and plasma samples were required for a participant to be included in this study, therefore, all participants who did not have both types of data were excluded from this study causing there to be small sample sizes for both groups (NC and SC). This becomes especially limiting when drawing conclusions about the association between sexual behaviors and HIV-1 seroconversion (Figure 2).

The participants of the MACS study, whose data is analyzed in this study, willingly chose to participate in the study which could introduce self-selection bias. Also, among the participants who seroconverted, only the ones who were well enough to attend their 6-month follow-up (visit 2) were able to provide sexual activity data and samples at this time point. This phenomenon, in this study, introduces survivorship bias and may have skewed the data to show lower inflammatory responses than there truly were.

The sexual behaviors analyzed in this study were self-reported which introduces self-reporting bias. Recall bias may be present as well given that participants were asked to recall the number of partners with whom they've performed the sexual behavior within the 6 months prior to each visit (male intercourse, receptive anal intercourse, and insertive anal intercourse) or in their entire lifetime (male intercourse ever).

4.2 Future Directions

As this study found no significant association between receptive anal intercourse and levels of inflammatory response biomarkers, it is necessary to explore other factors that may be involved in the pathway between sexual behaviors in MSM and HIV seroconversion. Therefore, the next step would be to explore the gut microbiome as one of those factors involved in the pathway. This would be done by analyzing fecal samples of the MSM evaluated in this study to investigate whether there is an association between their numbers of receptive anal intercourse partners and their gut microbiome composition and diversity.

In this study, we did not have the data available to evaluate how receptive anal intercourse affected local inflammation among the participants. We also did not analyze data on any active STD/STIs the participants may have had at the time their samples were collected. Both of these analyses would be beneficial future avenues of research.

A suggestion for expanding the scope of this study would be to look at both the number of partners with which a sexual behavior was performed and the number of times that sexual behavior was performed with each partner as measures for each sexual behavior.

5.0 Conclusions

The results of this study provide evidence to support that receptive anal intercourse is the sexual behavior most significantly associated with HIV-1 seroconversion in MSM. In addition, when evaluating the association between the number of receptive anal intercourse and plasma inflammatory response markers prior to and post- HIV-1 seroconversion, it was found that, overall, there was no significant association. These findings emphasize the importance of using condoms and other protective measures, as a means of HIV-1 prevention, when engaging in risky sexual behaviors such as anal intercourse.

6.0 Appendix A

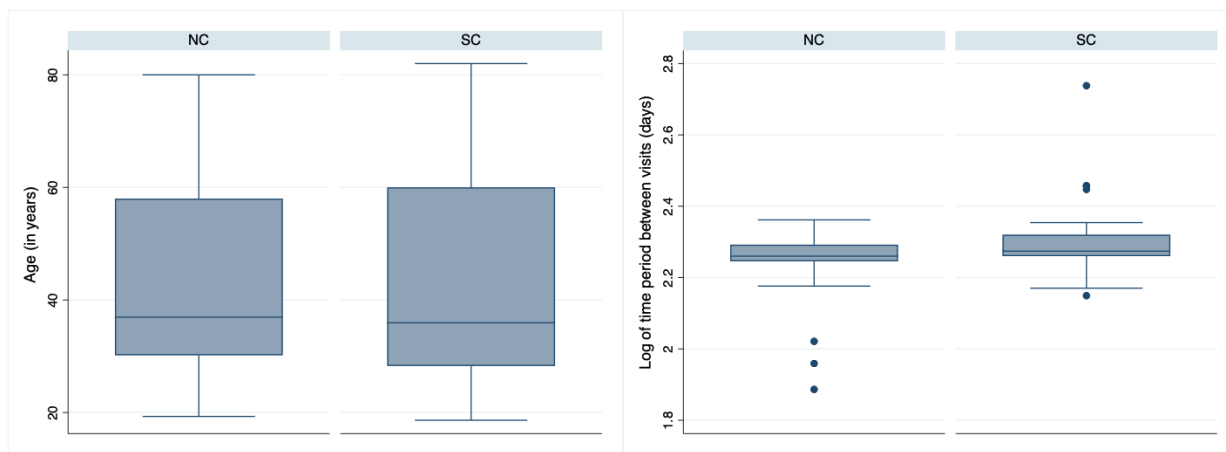


Figure 4. Distributions of “age” and “time period between visits” among participants

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